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MECHANISMS OF THE AGE-RELATED DIFFERENCES IN MORPHINE'S EFFECTS ON THERMOREGULATION, ANALGESIA, RESPIRATORY DEPRESSION AND THERMIC TOLERANCE IN RATS

The University of Arizona PH.D. 1982

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MECHANISMS OF THE AGE-RELATED DIFFERENCES IN MORPHINE'S EFFECTS ON THERMOREGULATION, ANALGESIA, RESPIRATORY DEPRESSION AND THERMIC TOLERANCE IN RATS

by

James Nelson McDougal III

A Dissertation Submitted to the Faculty of the

COMMITTEE ON PHARMACOLOGY AND TOXICOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by James Nelson McDougal III entitled Mechanisms of the age-related differences in morphine's effects on thermoregulation, analgesia, respiratory depression and thermic tolerance in rats.

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

David O. Johnson	May 28, 1982 Date
Richard L. Stouffer	May 28, 1982 Date
David A. Grelle	May 28, 1982 Date
Paul R. Murphy	May 28 1982 Date
Thomas F. Burks	May 28, 1982 Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

T. F. Burks Dissertation Director	June 8, 1982 Date
P. R. Murphy Dissertation Co-director	June 8, 1982 Date

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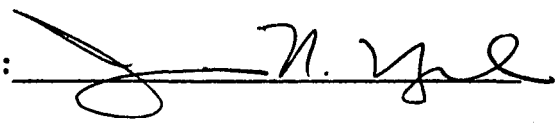
SIGNED: 

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ABSTRACT

Thermoregulatory, analgesic and respiratory depressive responses as well as tolerance to morphine were investigated in young (3 to 5 month), mature (10 to 12 month) and senescent (26 to 28 month) male Fischer 344 rats. The thermoregulatory system of senescent rats was not able to maintain body temperature in hot and cold environments as well as the thermoregulatory system of young rats. Additionally, senescent rats had basal rectal temperatures which were approximately one degree lower than basal temperatures in young rats. Subcutaneous morphine caused biphasic effects on body temperature ie. hyperthermia at low doses and hypothermia at high doses. Senescent rats were less responsive to the hypothermic effects of subcutaneous morphine than young rats, but this was not due to decreased subcutaneous blood flow or inability to lose heat. Morphine injections intracerebroventricularly showed no age-related differences. A two site model for the actions of morphine on thermoregulation was proposed and it was suggested that the age-related differences are due to changes in a non periventricular site. Previously reported increased lethality of intravenous morphine in aged rodents was shown to be due to decreased respiratory reserve rather than increased sensitivity to respiratory depression. Senescent rats were also found to acquire tolerance to the thermic effects of morphine less readily than young rats regardless of the

route of administration. Normal aging has been characterized as a decrease in adaptability, and it was suggested that senescent rats were less able to conpensate for the thermic effects of morphine as well as young rats. In order to determine the mechanisms of decreased adaptability, neurotransmitters proposed to be involved in thermoregulation were injected intracerebroventricularly in morphine tolerant rats. The results suggested a shift from catecholaminergic to cholinergic transmitters with aging.

INTRODUCTION

Geriatric Pharmacology

Studies of age-related changes in pharmacological response are becoming increasingly important because the number of elderly individuals in the U.S. population has increased. The percentage of the U.S. population over 65 years of age increased from 4 percent in 1900 to 11 percent in 1980 (Statistical abstract of the U.S., 1980). It was projected that by the year 2030 seventeen percent of the population will be over 65 (Vestal, 1978). Special populations, such as those individuals over 65 who are eligible for Veterans Administration benefits, will have an even greater rate of growth. It has been estimated that the proportion of adult male veterans over the age of 65 will increase from 26 percent in 1970 to 59 percent in 2000 (National Academy of Sciences, 1977).

Aged individuals, as a group, have more illness and take more medications than younger individuals. As the proportion of aged in the population increases, the economic impact of geriatric medicine could become staggering. Vestal (1978) estimated that the percent of drug expenditures for the elderly will increase from 25 percent of the national total in 1976 to 40 percent of the national total in 2030. According to Wilson, Lawson and Bravis (1962), of 200 consecutive admissions to a geriatric hospital in Scotland, 78 percent of the patients had 4 or more major diseases, 38 percent had 6 or more,

and 13 percent had 8 or more. One study has shown that hospitalized medicare patients were receiving an average of 10 prescription medications (Nithman, Parkhurst and Sommers, 1971). More than one disease encourages therapy with more than one drug, and thus the potential for adverse side effects or loss of efficacy due to multi-drug therapy is greatly increased. The incidence of adverse drug reactions has been reported to increase steadily with age, with patients between 70 and 79 years old having seven times as many adverse reactions as patients between the ages of 20 and 29 (Hurwitz, 1969).

Pharmacokinetics

The physiological changes associated with "normal" aging would be expected to be reflected as changes in pharmacokinetics. Among these physiological changes are a decrease in lean body mass and total body water, a decrease in serum albumin concentration, a decline in glomerular and tubular function in the kidney, diminished cardiac output, decreased liver blood flow and decreased cerebral blood flow (Vestal, 1978). These changes would be expected to alter both therapeutic effects and toxicity of drugs administered to the elderly.

Absorption. A decrease in stomach acid production with aging may interfere with drug absorption by a direct effect or by increasing gastric motility (Richey and Bender, 1977). Intestinal blood flow has been reported to decrease 40-50 percent with aging (Bender, 1965). This decrement in intestinal perfusion has the

potential to decrease absorption of a drug across the mucosal surface. Age-related decreases in the small intestinal absorption of xylose (Webster and Leeming, 1975), calcium (Ireland and Fordtran, 1973) and fat (Webster, Wilkinson and Gowland, 1977) suggest that similar decreases in absorption of drugs from the gastrointestinal tract may occur. However, studies of oral administration of drugs to old and young patients have not shown reduced gastrointestinal absorption with aging, and differences in blood levels or half-lives are generally suggested to have been due to changes in excretion or distribution (Triggs and Nation, 1975 ; O'Malley, Judge and Crooks, 1976). On the other hand, Bendkowski (1970) has reported unabsorbed tablets in the stools of aged patients.

Absorption of drugs from parenteral sites could be affected by changes in peripheral blood flow as reported by Bender (1965). In his summary of the literature, he found that systolic blood pressure increased and systemic flow decreased with aging. However, no age-related differences in percutaneous absorption of drugs have been reported. Leikola and Vartia (1957) concluded from their administration of penicillin G that there were no age-related differences in the rate of absorption from the intramuscular (gluteal) site.

Distribution. The "normal" aging process has been shown to result in physiological changes which alter the distribution of a drug. Changes in composition of the compartments into which drugs distribute as well as changes in the number of sites at which drugs

are bound, and therefore unavailable for metabolism, excretion or activation of receptors, have been shown to occur with aging. Lean body mass in proportion to body weight decreases with age (Forbes and Reina, 1970), and this appears to be due both to an increase in body fat (Novak, 1972) and to a decrease in total body water (Shock et al., 1963 ; Vestal et al., 1975). One would expect that these changes would cause higher blood levels of drugs that are distributed in total body water or lean body mass as well as accumulation and longer duration of action of lipid soluble drugs.

The amount of pharmacologically active or free drug may change with aging in highly protein-bound drugs. Although total serum proteins do not change with aging, serum albumin is reduced, and the globulin fraction is increased (Cammarata, Rodnan and Fennell, 1967). No age-related change in drug binding to serum proteins with aging has been found with phenobarbituric acid, benylpenicillin, diazepam, desmethyldiazepam, salicylate or sulphadiazine, but a reduction in binding has been reported with pethidine, phenylbutasone and phenytoin (Crooks, O'Malley and Stevenson, 1976 ; Vestal, 1978).

Metabolism. Age-related decreases in hepatic drug metabolizing capacity have been documented in laboratory animals (Kato and Takanaka, 1968), and several drugs appear to be metabolized more slowly in aged humans. An age-related decrease in hepatic blood flow has been shown (Bender, 1965 ; Geokas and Haverback, 1969) which could affect the rate of metabolism. In many cases it is hard to determine if the age-altered effect is due to changes in metabolism,

protein binding, volume of distribution or renal excretion. Antipyrine has been used as a model compound to investigate metabolic changes with aging; it is rapidly absorbed and completely metabolized in the liver but not bound to plasma proteins. Antipyrine has been shown to have a reduced clearance and prolonged half-life in the elderly (Liddell, Williams and Briant, 1975 and Vestal et al., 1975).

Excretion. Studies have shown an age-related decrement in glomerular filtration rate (Davies and Shock, 1950 and Rowe et al., 1976), a decrease in renal plasma flow (Bender, 1967), and a decrease in tubular function (Miller, McDonald and Shock, 1952). These changes in kidney function may be clinically significant with drugs such as antibiotics and amino glycosides where renal clearance is the major route of administration, and dosages should be reduced accordingly (Crooks, O'Malley and Stevenson, 1976; O'Malley, Judge and Crooks, 1976 and Vestal, 1978).

Pharmacodynamics

The "normal" aging process has been characterized as a progressive decrease in adaptability (Adelman, 1979), and many of the accompanying physiological changes could be expected to alter pharmacodynamics. Pharmacodynamics involves the site of action of a drug (ie. receptor), the connection of this site of action to the organelle or organ as a whole and the homeostatic capacity of the system containing the site of action. The study of pharmacodynamics begins where pharmacokinetics ends (with blood levels) and ends with

the production of the desired effect whether it be analgesia, vasodilation or immune suppression.

So far, studies of geriatric pharmacodynamics have been of only two types. Since the advent of radioligand binding studies, several investigators have looked at age-related changes in drug responsiveness at the receptor. Roth (1979) and Pradhan (1980) have reviewed radioligand binding with aging. In the other type of study a handful of investigators have indirectly looked at changes in the homeostatic capacity of the aged animal by investigating drug tolerance. Carney and associates (1980) have found that old rats acquire tolerance to phenobarbital more slowly than young rats.

Morphine Pharmacology

Morphine and other opiates have diverse effects on the central nervous system (CNS) and the gut. Among these actions are analgesia, decreased gastrointestinal transit, mood effects, respiratory depression, nausea, peripheral dilatation and drowsiness. Morphine's main clinically useful effect is analgesia; in fact morphine is the standard analgesic by which all new analgesics are judged. It is used in medicine for the relief of severe acute and chronic pain from pediatrics to geriatrics and can be administered subcutaneously, intravenously, intramuscularly and occasionally orally. Morphine and other narcotic analgesics are frequently used for surgical preanesthesia, relief of postoperative pain and the pain of terminal illness.

Pharmacokinetics

Morphine is rapidly absorbed from subcutaneous or intramuscular depots, the gastrointestinal tract or other mucous membranes. The volume of distribution of morphine is 3.2 liters per kilogram (Stanski, Greenblatt and Lowenstein, 1978), and it is approximately one third protein-bound (Olsen, Bennett and Porter, 1975). Free morphine rapidly leaves the blood and accumulates in parenchymatous tissues such as kidney, lung, liver and spleen (Jaffe & Martin, 1980), but only a small amount of morphine crosses the blood-brain barrier (Oldendorf et al., 1972).

Morphine administered to humans and rodents is metabolized primarily by glucuronide conjugation, at the phenolic hydroxyl group, in the liver, but a small amount of N-demethylation occurs in man (Jaffe and Martin, 1980). First-pass metabolism greatly reduces the potency of oral morphine, and enterohepatic circulation of both morphine and morphine glucuronide occurs. Morphine is primarily excreted by glomerular filtration, although 7 to 10 percent of administered morphine may appear in the feces (Jaffe and Martin, 1980). The plasma half-life of parenteral morphine is 2 to 3 hours (Berkowitz, 1976).

Pharmacodynamics

The mechanism of action of morphine and other opiates is only partially understood. However, less than a decade ago the discovery of the opiate receptor (Pert and Snyder, 1973; Simon, Hiller and Edelman, 1973 ; Terenius, 1973) and the sequencing of endogenous

enkephalins (Hughes et al., 1975), as well as the use of relatively specific antagonists such as naloxone, have facilitated the study of opiate pharmacodynamics. It is assumed that morphine and other exogenous opiates act by mimicking the effects of endogenous opiates at the opiate receptors on cell membranes. The major endogenous opiates, enkephalins and beta-endorphin, function as neurotransmitters, neuromodulators or neural hormones.

Endogenous opiate peptides have been shown to be non-uniformly localized in several areas of the body which are concerned with regulation. Enkephalin-like peptides are widely distributed in several areas of the CNS (notably laminae I and II of the spinal cord, brain periaqueductal gray, raphe nucle, locus ceruleus, globus pallidus and median eminence) as well as the myenteric plexus and other areas of the gut (Hughes, Kosterlitz and Smith, 1977). Beta-endorphin-like activity is localized to the hypothalamus and pars intermedia and pars nervosa of the pituitary gland (Snyder, 1978; Beaumont and Hughes, 1979).

Receptors or sites of action for the endogenous opiates have been thoroughly investigated with the use of a wide variety of species, drugs and techniques. Many groups (Martin et al., 1976; Lord et al., 1977; Cowan, Geller and Adler, 1979; Wüster, Schulz and Herz, 1980 ; Lee and Smith, 1980) have proposed different methods of classification of opiate receptors; however one point of agreement is opiate receptor heterogeneity. In some classic studies on the chronic spinal dog, Martin and associates (1976) identified three

types of receptors (mu, kappa and sigma). The mu receptor, with morphine as prototype agonist, was shown to cause miosis, bradycardia, hypothermia, depression of nociception and response to stimuli. The kappa syndrome induced by ketocyclazocine was characterized by miosis, sedation and depression of the flexor reflex. SKF-10,047, the sigma agonist, induced mydriasis, tachypnea, tachycardia and mania. The effects of all three drugs were antagonized by naltrexone, but they were suggested to the the result of activation of different receptors because of the lack of cross-tolerance and the dose of naltrexone required to antagonize the effects.

Since that study several modifications to opiate receptor classification have been suggested. Lord and associates (1977) suggested a fourth type of receptor after finding that the enkephalins were more potent than morphine in the mouse vas deferens. They proposed that this enkephalin activity was mediated by a receptor they called delta. A fifth type, epsilon, was suggested when it was found that the rat vas deferens contained a receptor that was very sensitive to beta-endorphin but not morphine or the enkephalins (Schulz et al., 1979). Due to the diversity of species and techniques used to categorize opiate actions, a moderate degree of confusion exists concerning the nature of the heterogeneity of opiate receptors. Lee and Smith (1980) have recently suggested that the majority of the previously mentioned phenomenon could be explained by just two receptors: one for alkaloids and one for

enkephalins. They suggested that beta-endorphin activates both. Morphine has been shown to act primarily on the mu receptor to affect the release of acetylcholine, norepinephrine, substance P and dopamine (Snyder, 1978; Beaumont and Hughes, 1979) with subsequent functional effects including analgesia and changes in thermoregulation.

Thermoregulation. Although they are not clinically useful, the effects of morphine on thermoregulation in some species are dramatic and fascinating. Body temperature in the human falls slightly after a single therapeutic dose of morphine and is elevated by chronic dosage (Jaffe and Martin, 1980). The effect of morphine on body temperature in laboratory animals has been reviewed by Ary and Lomax (1979) and Burks and Rosenfeld (1979a). The thermic effects of opiates have been shown to be due primarily to actions on the preoptic and anterior areas of the hypothalamus (Lotti, Lomax and George, 1965 ; Baldino, Beckman and Adler, 1980) and the spinal cord (Rudy and Yaksh, 1977). In the rat, small subcutaneous doses of morphine (1-10 mg/kg) cause hyperthermia, and larger doses cause hypothermia. Morphine-induced hyperthermia is a result of slightly increased heat production and decreased heat loss, accompanied by increased sympathetic outflow (Hermann, 1942). Morphine-induced hypothermia is due to decreased heat production and increased heat loss (Reynolds and Randall, 1957) and/or loss of thermoregulatory control (Clark, 1979). Morphine applied iontophoretically to temperature sensitive preoptic/anterior hypothalamic neurons resulted

in excitation of warm-sensitive cells and inhibition of cold-sensitive cells, and these effects were blocked by naloxone (Baldino, Beckman and Adler, 1980). The hypothermic response to subcutaneous morphine has been shown to be mediated by serotonin and norepinephrine but not by dopamine (Burks and Rosenfeld, 1979b). However, dopamine has been shown to be involved in the thermoregulatory pathway (Cox and Lee, 1980) and morphine withdrawal hypothermia (Cox, Ary and Lomax, 1976).

Analgesia. The effects of opiate analgesics are to increase the patient's ability to tolerate pain although the perception of pain may not be greatly altered. Morphine probably exerts its analgesic effects by acting at several sites. Electrical stimulation of the periaqueductal gray or dorsal raphe nucleus has been shown to produce analgesia (Giesler and Liebeskind, 1978) probably at least in part by a descending serotoninergic system which modulates ascending pain transmission in the dorsal horn of the spinal cord via an enkephalin containing neuron (see Kelly, 1981). However, opiates also suppress nociceptive withdrawal reflexes below the level of transection of the spinal cord (see Jaffe and Martin, 1980). Hypothalamic stimulation also causes analgesia probably by an endorphin rather than by a serotonergic mechanism (Hosobuchi et al., 1979).

Respiratory Depression. The limiting factor to the clinical use of morphine as an analgesic is respiratory depression which can result in death.Morphine depresses respiratory rhythmicity, rate and

tidal volume by actions in the central nervous system. The respiratory depressant effect of a therapeutic dose of morphine in humans lasts 4 to 5 hours and can be produced by doses which do not cause mood changes. Morphine acts by depressing the sensitivity of medullary respiratory centers to carbon dioxide tension and electrical stimulation (Florez, McCarthy and Borrison, 1968). Goode, Rhodes and Waterfall (1979) have shown that 4 mg/kg of morphine caused respiratory depression in the rat.

Tolerance and Dependence

Tolerance is defined to be present when, after continued administration, a given dose of a drug produces a decreased effect or, conversely, when a larger dose is required to get the initial effect. Tolerance to the analgesic, respiratory depressant, thermoregulatory, sedative and euphoric effects of morphine develops with continued use, but a significant degree of tolerance does not develop in clinical use to the miotic and constipative effects of opiates (Jaffe, 1980). The mechanisms involved in opioid tolerance are not precisely known; however, tolerance can be considered a homeostatic compensation to an action of a drug. A specific example of tolerance would be decreased depression of spinal reflexes with continued administration of morphine. Cross-tolerance is the acquisition of tolerance to one drug due to the administration of another drug. The investigation of the existence of cross-tolerance is used as a tool to determine if two drugs are having their effects at the same receptor or in a common pathway.

Unlike tolerance, which is demonstrated by drug administration, dependence is demonstrated by drug deprivation. Dependence is the result of the now inappropriate homeostatic compensation for the drug effect. A specific example of dependence would be hyperexcitable spinal reflexes after cessation of morphine administration in the tolerant animal. The existence of dependence may also be determined by an operant response leading to drug self-administration. When tolerance and dependence are present, cessation of drug administration may result in a withdrawal syndrome, which is a condition of varying severity occurring during the time the now inappropriate homeostatic compensation is returning to normal. The onset of withdrawal is thought to depend on the removal of the drug from its site of action. The opiate antagonist, naloxone, is frequently used to precipitate opioid withdrawal, presumably by displacing opiates from their receptors (Jacob, 1974). In rats, precipitated opioid withdrawal is characterized by jumping, teeth chattering, irritability to touch, diarrhea, chewing, wet dog shakes and weight loss (Collier, Francis and Schneider, 1972).

Thermoregulation

In mammals, the maintenance of body temperature $\pm$ 0.6 ^{o}C under diverse conditions involves an intricate temperature sensing system, a hypothalamic "thermostat" and integrated control of heat loss and heat gain mechanisms. Regulation of body temperature has been reviewed by Hammel (1968), Bligh (1979), Gale (1973) ; Satinoff (1978).

Anatomy

The "eyes" of the thermoregulatory system are its temperature sensitive neurons (reviewed by Hensel (1974) and Schmidt (1978)). These neurons transduce or convey information about temperature in the skin, spinal cord, visceral organs and brain stem to CNS thermoregulatory centers (Pierau and Wurster, 1981). Three types of neurons in the brain, responsive to thermal stimulation have been identified (Poulos, 1981). Two of these types respond only to thermal stimuli and the third responds to mechanical as well as thermal stimuli. Cold receptors increase their firing frequency with cooling, and warm receptors increase firing rate via temperature dependent ionic conductance when warmed. Both cold and warm epidermal receptors transmit thermoreceptive information to the CNS via myelinated A delta and non-myelinated C fibers respectively (Dodt and Zotterman, 1952).

Thermoresponsive cells have also been found in several areas of the CNS including the preoptic and anterior area of the hypothalamus (POAH), caudal hypothalamus, septal nuclei, midbrain-pons, medulla oblongata, spinal cord, thalamus and cerebral cortex (see Reaves and Hayward, 1979). The importance of thermoresponsiveness in these latter areas is not fully understood, and it is thought that the anterior hypothalamic area is the primary area for sensing brain temperature (Kupfermann, 1981). Electrical, chemical and thermal stimulation has implicated the preoptic, anterior and posterior areas of the hypothalamus with integration of

afferent information and initiation of the appropriate autonomic response (Reaves and Hayward, 1979).

Autonomic Regulation

Mammals have an amazing ability to unconsciously maintain homeothermy within a very narrow range in all types of environments. A nude human can be exposed to ambient temperatures as high as 60 °C and as low as 13 °C and still maintain euthermy (Guyton, 1976). In order to maintain a normal body temperature of 37 °C, an organism exposed to such extremes must lose heat to a very much hotter environment and avoid losing heat to a very cold environment. These integrated autonomic feats can best be understood by looking at the mechanisms responsible for heat production and loss.

Heat Production. Basal heat production (a byproduct of normal metabolism) is such that mammals unable to give off heat would become hyperthermic and die. Fifty-eight Kcal absorbed from the gastrointestinal tract would raise the body temperature of a 70 Kg man 1 °C if he were unable to lose heat (Brengelmann, 1973). However, basal heat production is normally balanced by unavoidable heat loss. Heat production can be increased above the basal rate by shivering, nonshivering thermogenesis, and increased physical activity.

Shivering thermogenesis can result in a rapid increase in metabolic heat production of two to five times above basal (Jansky, 1979). Shivering is characterized by rhythmic involuntary movements of opposing muscle groups, and since no mechanical work is accomplished all energy released by muscular contractions is in the form

of heat. Shivering is controlled by a dorsomedial portion of the posterior hypothalamus near the wall of the third ventricle, which is normally inhibited by the POAH but is activated by thermosensors in the skin and cord (Stuart, 1961). Efferent pathways down the cord to ventral horn motorneurons do not cause the actual shaking but mediate an increase in muscle tone in all parts of the body. Heat production is raised even before shivering occurs but once the muscles reach a critical tone shivering begins, probably from feedback oscillation of the muscle spindle stretch mechanism (Stuart, 1966). It has been estimated that 48 percent of the heat generated by shivering is retained in the body (Hardy, 1961).

Nonshivering thermogenesis, sometimes called chemical thermogenesis, is the production of heat during metabolism. Two types of metabolic heat production can be distinguished. The first is heat produced by an organism resting in a post-absorptive state in a thermoneutral environment, and has been called "obligatory" themogenesis. The second is heat produced in response to cold and is called "regulatory" or "facilitative" thermogenesis (Jansky, 1973). "Obligatory" themogenesis is slow to change and is mostly controlled by thyroid hormones. In contrast "regulatory" thermogenesis can be called upon more rapidly and is controlled by catecholamines, thyroid hormones and glucocorticoids (Horowitz, 1979 ; Marques et al., 1981). "Regulatory" thermogenesis can be increased to two or three times the level of "obligatory" thermogenesis (Jansky, 1979).

Heat Loss. Heat is lost from animals by radiation, conduction and evaporation. Convection can affect both conduction and evaporation. Fifty to sixty percent of the heat loss by a nude person at room temperature is via radiation (Brengelmann, 1973). Radiant heat is transferred from a warm object to cooler ones and therefore the temperature difference between an animal and its environment determines both the direction and the magnitude of heat exchange. Clothing and fur reduce heat loss significantly.

Heat can be lost by conduction to both the air and objects with which an animal is in contact. Energy is transferred by conduction only when there is direct contact, and 15 percent of the heat lost by a nude person is due to conduction, mostly to air (Guyton, 1976). Heat loss by conduction is increased by convection and decreased by about one-half by clothing or fur.

Evaporative heat loss can be categorized as "insensible" or uncontrollable and "sensible" or controllable heat loss. "Insensible" heat loss from skin and lungs rids man of 12 to 18 Calories per hour (Mather, Nahas and Hemingway, 1953 ; Brengelmann, 1973). "Sensible" heat loss or sweating is under autonomic control and is the major method by which humans lose heat in warm environments. Convection can aid evaporation and increase "sensible" heat loss. Panting is the most effective method for evaporative heat loss in furred mammals (Hammel, 1968).

Animals can greatly modify heat loss by the above mechanisms by controlling peripheral blood flow. When heat loss is desirable,

vasodilation of vessels in the skin and extremities allows equivalent skin and core temperatures and therefore maximum potential for heat loss (Grayson and Kuehn, 1979). In an environment in which heat loss is undesirable, sympathetic peripheral vasoconstriction will maintain core temperature and allow peripheral temperature to fall thereby reducing core heat loss (Grayson and Kuehn, 1979).

Behavioral Regulation

All animals capable of thermoregulation partially adjust their body temperature behaviorally. Ectotherms only have behavioral thermoregulatory means available to interact with their thermic environment. Behavioral thermoregulation, in contrast to autonomic regulation, implies awareness or perception of the relationship between ambient and body temperatures. Behavior among mammals which influences core temperature may range from putting on a sweater and moving closer to the fire to the spreading of saliva on the fur and postural adjustments to facilitate heat loss (sprawling).

Behavioral and autonomic thermoregulatory activities have been shown to be anatomically separate by lesion studies. Rats with preoptic lesions and which were unable to regulate autonomically, would manipulate their environment by bar pressing to turn on a heat lamp or fan (Lipton, 1968 ; Carlisle, 1969). Lateral hypothalamic lesions abolished operant thermoregulatory responses while leaving autonomic responses intact (Satinoff and Shan, 1971). According to Satinoff (1978) however, these functionally and neuroanatomically

separate responses are thoroughly integrated in a hierarchical control system.

Thermopharmacology

The classic method of studying CNS control of thermoregulation has been to use drugs and/or thermal stimulation. Investigations in thermopharmacology embrace a large variety of thermoactive substances with the purpose of uncovering the intricacies of thermoregulatory control, rather than of development of drugs for clinical intervention. With the exception of antipyretics, thermopharmacology in the clinic is presently non-existent.

Neurotransmitters. Models of thermoregulatory control are based on known neurotransmitters in hypothalamic control of thermoregulation (Bligh, 1979 ; Myers, 1975). These transmitters cause measurable changes in body temperature when injected centrally (see Myers, 1974 ; Cox and Lomax, 1977). Cholinergic agonists administered centrally to rats have been reported to cause both hypothermia (Beckman and Carlisle, 1969; Kirkpatric and Lomax, 1970 ; Poole and Stephenson, 1979) by activating heat loss mechanisms and hyperthermia (Avery, 1970). According to Crawshaw (1979), acetylcholine has a role in thermoregulation in many species; however, due to interactions and complexity, its exact role is still to be discovered. Norepinephrine has caused predominantly hypothermia in rats (Feldberg and Lotti, 1967 ; Poole and Stephenson, 1979) an effect which may be blocked by the alpha adrenoreceptor antagonist, phentolamine (Bruinvels, 1975). According to Bruinvels

(1979) the most likely involvement of norepinephrine in the rat is activation of heat loss pathways. 5-HT causes hypothermia in the rat although 5-HT's role in thermoregulation is not precisely known (see Jacob and Girault, 1979). Dopamine causes hypothermia in the rat and has been suggested by Cox (1979) to mediate heat loss.

Peptides. With the present focus of attention on neuropeptides as transmitters or neuromodulators, several peptides which affect thermoregulation have been found. Bombesin is the most potent peptide found with thermoregulatory effects. Bombesin has been shown to produce hypothermia due to an interference with "regulatory" thermogenesis (Brown, 1981). An analog of somatostatin (OTD8-SS) produces hyperthermia (Brown, Ling and Rivier, 1981) and inhibits bombesin-induced hypothermia. Beta endorphin and met-enkephalin act much like morphine in laboratory animals, ie. both hyperthermia and hypothermia can be produced depending on dose and restraint (Clark, 1981). There has been speculation that endogenous opiates are involved in thermoregulation as transmitters or neuromodulators. Lal, Miksic and Smith (1976) have shown that naloxone pretreatment prevented a conditioned hyperthermic response to morphine, and Holaday et al. (1978) have suggested that endorphins function in heat adaptation. Adrenocorticotropin (ACTH) and alpha-melanotropin cause hypothermia in rabbits by inhibiting heat production (Lipton, Glyn and Zimmer, 1981).

Drugs. A complicated control system such as thermoregulation would be expected to be susceptible to perturbation by drug effects; it may be that every centrally active drug given in high enough doses will affect body temperature. Because of the well-regulated control of body temperature via negative feedback and the importance of competent thermoregulation, drugs would be expected to have only transient effects. Many drugs have predictable effects on thermoregulation due to their effects on neurotransmitter levels or neurotransmitter-mimetic effects. Other drugs which cause either CNS depression or excitation would be expected to have predictable effects on body temperature.

Pyrogens have been shown to cause an increase in body temperature by the release of an endogenous pyrogen called leucocyte pyrogen from polymorphonuclear leucocytes (Atkins, 1960). Allen (1965) found that iodinated leucocyte pyrogen injected systemically was found to be concentrated in the hypothalamus but not other parts of the brain. It is generally accepted that pyrogens act by elevating the hypothalamic set point (Cooper, Veale and Pittman, 1979). Leucocytic pyrogen may have its effects by increasing synthesis and release of prostaglandins from the hypothalamus (see Feldberg and Milton, 1973). Prostaglandin-like activity was reported to be released into the cerebrospinal fluid (CSF) of unanesthatized cats by bacterial pyrogen (Feldberg and Gupta, 1972). Both prostaglandin-induced and pyrogen-induced fever are abolished by antipyretic agents .cp6 such as acetominophen, indomethacin, aspirin

and sodium salicylate which inhibit prostaglandin synthesis (see Feldberg and Milton, 1973).

General anesthetic-type drugs such as pentobarbital, chloralose, thiopental, ether and ethanol cause hypothermia due to a decrease in muscle tone and heat production as well as vasodilation (see Lomax, 1970). Hypothermic agents which have less dramatic hypnotic effects may also produce their effects by CNS depression. Chlorpromazine and other phenothiazines cause hypothermia and occasionally hyperthermia in patients. This is probably due to a multitude of effects on metabolism, peripheral and central actions (see Borbely and Loepfe-Hinkkanen, 1979). Tricyclic antidepressants also cause a slight hypothermia by a more specific depletion of catecholamines (see Loskota and Schonbaum, 1979).

Aging and Morphine

Very few studies have compared the effects of morphine in old and young humans or laboratory animals. Due to their design, all of these previous studies have looked at age-related differences rather than age-related changes. In addition, most studies which have purported to investigate whether aging affects morphine responses have compared young animals to adults rather than truly aged animals. Only a few investigators have used laboratory animals which are comparable in stage of life to human geriatric population.

Thermoregulation

Only one study has investigated age-related differences in the thermic effects of morphine. Spratto and Dorio (1978) administered morphine (10, 30 and 50 mg/kg i.p.) to 1.5, 6 and 10 month old male Sprague-Dawley rats. They measured rectal temperatures hourly by intermittent probing and they found that the hyperthermic dose (10 mg/kg) caused the least hyperthermia in the 1.5 month group. They also reported that the 10 month group had less hypothermia with 50 mg/kg than the other two groups, and that the oldest rats had the longest delay to the peak response with the two lowest doses of morphine. Spratto and Dorio concluded that age altered the thermic response to morphine; however, no pattern of change was detected. Therefore, the effect of age on the thermoregulatory response to morphine has not been determined.

Analgesia

Age-related differences in analgesic effects of morphine have been investigated in patients and laboratory animals. Two studies of patients with postoperative pain (Bellville et al., 1971 and Kaiko, 1980) have shown that the patient's assessment of pain relief from morphine was directly related to age. Duration of relief from pain was shown to be greater in aged patients than in young patients (Kaiko, 1980).

Studies with laboratory animals have generally shown age-related differences in analgesic response. The analgesic response to morphine is greater in developing rats than in adults (Johannesson

and Becker, 1973). They measured analgesia to morphine (0.5 to 15 mg/kg s.c.) in male and female 20-42 days old and adult Sprague-Dawley rats using the hot plate technique and found that 7.5 to 10 times greater doses were required to elicit the same analgesic response in 42 day old and adult rats than in the developing ones. It has been reported that to produce equianalgesic responses to foot shock vocalization in 4 and 12 week old Sprague-Dawley rats the dose had to be reduced from 3 mg/kg to 2.3 mg/kg (s.c.) in the latter group (Nozaki et al., 1975). Johannesson and Becker (1973) found that by the time rats were 42 days old their responses were the same as those in the rats they referred to as adults (estimated to be 3 months old by weight). In contrast, Spratto and Dorio (1978) found that 1.5 month male Sprague-Dawley rats were more sensitive to morphine (5 mg/kg i.p.) analgesia measured by tail-flick than 6 or 10 month old rats; however, these differences disappeared at 7.5 mg/kg. Saunders and associates (1974) have shown using foot shock vocalization that 10 month male Cox/Sprague-Dawley rats are more responsive to morphine (5 to 14 mg/kg i.p.) than three month old rats. Thus it appears that developing rats are more sensitive to the analgesic effects of morphine than developed rats, although older rats (10 month old) may start to become more sensitive again. The only study using animals comparable to the geriatric population showed that old C57Bl/6J mice (28 months old) were less sensitive to increased tail flick latency due to morphine (5 mg/kg i.p.) than young mice (3 months old) (Webster, Schuster and Eleftherio, 1976).

Morphine analgesia, as assessed by patients, has been shown to be increased in the geriatric population; however, in laboratory rodents the picture is not clear.

Respiratory Depression

Spratto and Dorio (1978) have investigated age-related differences in respiratory depression in rats by infusing morphine at a constant rate (20 mg/min) until cessation of respiration. The dose of morphine necessary to produce cessation of respiration was greater in 1.5 month old rats than it was in 6 or 10 month old rats. Brain and plasma levels 10 seconds after cessation of respiration were also higher in the immature rats. Chen and Robbins (1943) showed that the lethal dose (LD_{50}) of morphine in rats decreases between 1.5 and 24 months of age. Meperidine, which depresses respiration in young normals to the same extent as an equianalgesic dose of morphine (Jaffe and Martin, 1980), has been shown to be a more effective respiratory depressant in elderly patients (Mather et al., 1974).

Pharmacokinetics

Berkowitz and associates (1975) found that early (2 minute) levels of morphine (10 mg/70 kg) in anesthetized surgical patients were correlated with age but that the serum half-life showed no age-related differences. In laboratory animals, age-related differences in brain and serum levels have been reported. Developing rats (20 days old) were reported to have twice the brain level but similar plasma levels 40 minutes after administration of morphine (5 mg/kg

s.c.) (Johannesson and Becker, 1973). Spratto and Dorio (1978) reported lower brain and serum levels over a four hour period in 1.5 versus 6 and 10 month old rats after administration of 30 mg/kg (i.p.) of morphine. Serum and brain levels were reported higher in old rats (20 month old) when compared to young (2 month old) rats at both 1 and 6 hours after morphine (5 mg/kg i.v.) (Berkowitz, 1974). In mice (Webster et al., 1976) the serum half-life of morphine was reported to be longer in 24 month old males compared to 6 month old males.

Tolerance and Dependence

One study (Nozaki et al., 1975) examined tolerance and dependence in developing rats. They found that 4 week old rats developed tolerance to repeated injections of morphine more rapidly than 12 week old rats, but that naloxone-precipitated withdrawal (5 mg/kg s.c.) resulted in the same fractional change in body weight after 23 days of morphine administration. However, no studies of morphine tolerance or dependence in old rats have been reported.

Statement of the Problem

Although morphine has been used therapeutically for over 100 years and opium use began centuries before that, very little is known about how morphine interacts with the human altered by aging or senescence, despite the fact that aged individuals may take a disproportionate share of opiates. The purpose of this investigation was to use and validate the senescent rat model in the elucidation of

age-related differences in morphine pharmacology and the mechanisms involved. The specific aims were to determine if thermoregulatory effects and tolerance/dependence as well as analgesia and respiratory depression induced by morphine show age-related differences in rats. Mechanisms of the differences observed were explored by dose-response analysis, cross-tolerance studies, temperature stress, and varying the routes of administration.

METHODS

Experimental Design

All experiments were designed and carried out such that treatments were as identical as possible. The only desirable difference between the groups was the age of the rats. Seven to ten rats were used in each age group. Dependent variables included measurements such as tail-flick latency, respiratory frequency, displacement of rectal temperature from baseline and morphine immunoreactivity in the serum. Each rat was naive to treatment except in tolerance studies.

Repeated measurements were necessary in the thermoregulation studies for determination of tolerance acquisition. One dose of a drug was given three times to the same rat; first when naive, again three days later and finally three days after morphine pellet implantation. A paired t-test, using the naive response as a control was used to determine if tolerance had been acquired by the second or third doses.

Drug injections in all thermoregulatory experiments were accomplished at the same time each day (1100 to 1200) to avoid diurnal variation, and the thermic responses of all age groups were measured during the same experimental session. Measurements of respiratory depression and tail-flick were made one rat at a time with alternation of age groups, i.e. first a young rat, then a mature rat and then a senescent rat.

Fischer 344 Rats

Rats used in these experiments were male Fischer 344 rats of three age groups. Some of these rats were graciously provided by the National Institute on Aging under their pilot/predoctoral support program. Fischer 344 rats are descendents of a first mating in Sept, 1920. These rats, from a highly inbred small strain, are cesearean-originated and pathogen barrier sustained at Charles River Breeding Laboratories. Once delivered to the animal facilities, rats were housed individually in hanging cages at an ambient temperature of 20-21 ^{o}C, fed laboratory chow ad libitum and maintained on a 12 hour light/dark cycle. The mean time rats were maintained in the animal facilities prior to use was approximately 3 weeks.

Lifespan

Chesky and Rockstein (1976) accumulated lifespan data for 572 male non-breeding Fischer 344 rats over a 5 year period. They found that the mean life-span of these rats was 21.1 months, and only 25 percent of them survived to 26 months of age. However, when Fischer rats were raised behind a barrier, they had a mean survival time of 29 months and a maximum of 35 months (Coleman et. al., 1977).

Age groups used in these studies were young (3 to 5 months), mature (10 to 12 months) and senescent (26 to 28 months). An attempt to compare the lifespan of a rat with that of a human can be made by comparing the time of puberty and longevity. The age at which rats can have a litter is 13 weeks (Chesky and Rockstein, 1976) and the gestation period is 21 days, which gives an estimate of puberty of 10

weeks. The age of puberty for human males in the U.S. is twelve to eighteen years (Statistical Abstract of the U.S.: 1980). This comparison places the young rats equivalent in age to human late teenagers. Mature rats would be comparable to humans in their thirties and senescent rats would be analgous to septuagenarians.

Pathology

Fischer 344 rats have been known to have a high incidence of testicular interstitial cell tumors (Jacobs and Huseby, 1967). Coleman and associates (1977) have recently investigated several pathological changes during aging in male Fischer rats. They found that there are several neoplasms which have greater than 10 percent incidence: testicular interstitial cell tumor (66.2 percent), mononuclear cell leukemia (16 percent) and pituitary chromophobe adenoma (14.7 percent). They also found a high positive correlation between age and renal disease. Other findings were an age-related decrease in total serum protein and albumin and an age-related increase in alpha-1-globulin and cholesterol.

Drugs

Drugs used were morphine sulfate (Merck Chem. Co., Rahway NJ), U.S.P. ethyl alcohol (U.S. Industrial Chem. Co., New York NY), ketamine hydrochloride (Ketalar, Parke-Davis, Morris Plains NJ), ethyl ether (MCB Manuafacturing Chemists Inc., Cincinnati OH), naloxone hydrochloride (Endo Laboratories, Garden City NJ), acetylcholine chloride (Calbiochem, San Diego CA), dopamine

(3-hydroxytyramine hydrochloride) and norepinephrine ((-)arterenol hydrochloride) (Sigma Chem. Co., St. Louis MO).

Peripheral Administration

Drugs administered subcutaneously (s.c.) and intraperitoneally (i.p.) were dissolved in bacteriostatic sodium chloride (Abbott Laboratories, North Chicago IL) and injected in a volume of 1 ml/kg of body weight. Drugs administered intravenously (i.v.) were dissolved in bacteriostatic saline and injected in a volume of 0.5 ml/kg. Drugs administered orally (p.o.) and intracerebroventricularly (i.c.v.) were dissolved in sterile water (Abbott Laboratories). Drugs given p.o. were in a total volume of 1 ml and drugs given i.c.v. were in a total volume of 5 microliters. Injections were made with a 25 ga x 5/8 in. needle into the lower left abdominal quadrant and on the dorsal midline at the level of the scapulae for i.p. and s.c. injections respectively. Intravenous injections were given with a 26 ga x 1/2 in. needle into the dorsal vein of the penis, either with or without (thermoregulation studies) light ether anesthesia. Oral administration was accomplished by gastric intubation with a 10 cm 16 gauge feeding needle.

Intracerebroventricular Administration

Three days prior to i.c.v. drug administration, rats were anesthetized with ketamine and guide cannulas were implanted above the left lateral ventricle (3 mm posterior to the saggital suture and 3 mm lateral to the coronal suture). Cannulae were anchored with

dental acrylic and one 00 x 1/8 in. flathead screw (J.I. Morris Co., South Bridge MA). The guide cannulas were fashioned from 22 ga needles and projected to a depth of 1 mm into the skull thus avoiding tissue damage. At the time of the i.c.v. injection, the unanesthetized rats were gently held and the drug was injected slowly into the ventricle through a 30 ga x 1/2 in. needle (Figure 1). Injection placement was confirmed visually at the time of sacrifice by dye injection using the same injection technique.

Thermoregulation

Rats were brought into the laboratory from the animal facility, weighed, marked, placed in the wire-mesh restrainer and probed with thermistors 3 to 4 hours before drug injection to allow time for them to acclimate to restraint. Temperature was measured using thermistor probes (Yellow Springs Inst. #401, Yellow springs, OH) inserted 6 cm into the rectum and gently secured to the base of the tail with 1/2 inch porous adhesive tape (ZONAS, Johnson and Johnson, New Brunswick NJ). At the time of injection, each rat was gently removed from the restrainer, injected while being held, and then returned to the restrainer. Rectal temperature for each rat was manually recorded at 15 minute intervals for at least one hour before and four to six hours after injection, using a tele-thermometer (Yellow Springs Inst. 46TUC) which has accuracy of $\pm$ 0.15 ^{o}C and readability of $\pm$ 0.05 ^{o}C.

Figure 1. Rat receiving i.c.v. injection. Cannula implantation occurred 72 hours prior and unanesthetized rat is gently held for the injection in the lateral ventricle.

Restraint

A wire-mesh restrainer (Figure 2) of our own design and fabrication was used to restrain different sized rats in a comparable manner. This restrainer also avoided the heat and humidity accumulation characteristic of the commonly used plastic restrainer. The apparatus consisted of an L shaped frame of 12 mm angle-iron on 9 cm legs. The frame supported a base and back of 1 mm galvanized expanded metal which was 124 cm wide x 30 cm deep and high. The base supported ten adjustable clamshell-type restrainers, made of galvanized hardware cloth, which were gently folded over the rat and fastened. The rat's tail and thermistor probe lead wire protruded through a small opening in an aluminum back which was fastened to the base with bolts. The interior length of the restrainer was 15 cm and the width at the base was 7.5 cm.

Data Expression

Deviations of rectal temperature from baseline were determined at each 15 minute time point by subtracting each rat's rectal temperature from baseline which was defined as the mean of the four 15 minute temperatures prior to drug injection. Thermal response indices (TRI) (Clark and Cumby, 1978) were calculated for each rat during the period of interest after injection. TRI is an integrated measurement of the area of the deviation of rectal temperature from baseline (Figure 3), and one TRI unit is equivalent to a 1 ^{o}C change from baseline of 1 hour duration. Both a negative TRI, from rectal temperatures less than baseline, and a positive TRI, from

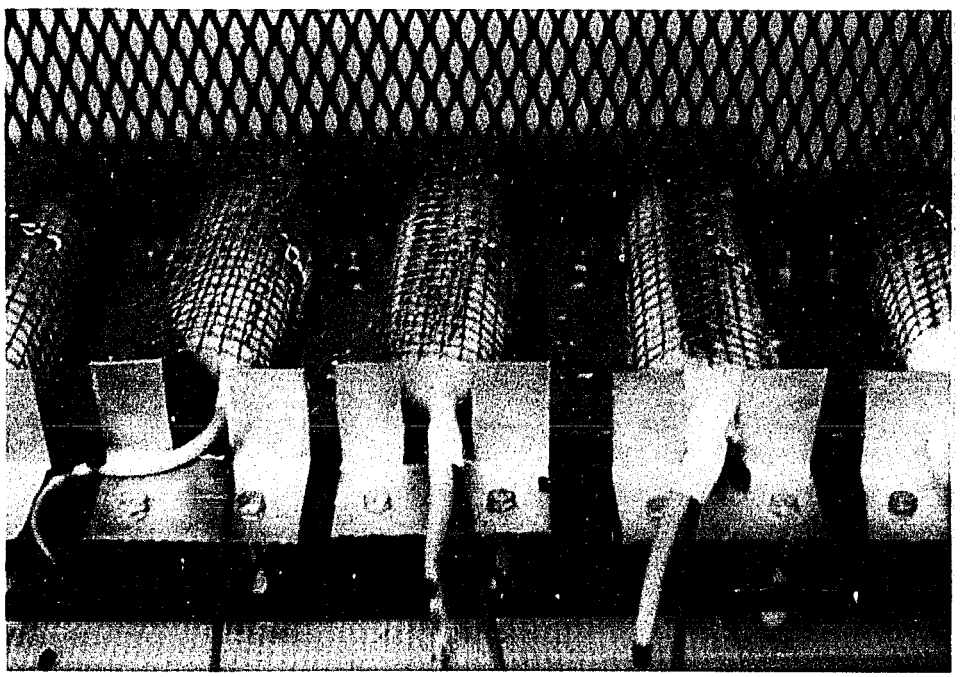

Figure 2. Rats in wire-mesh restrainer. Rats were placed in the locally manufactured restrainer so that rectal temperatures could be measured over a period of 4 to 6 hours.

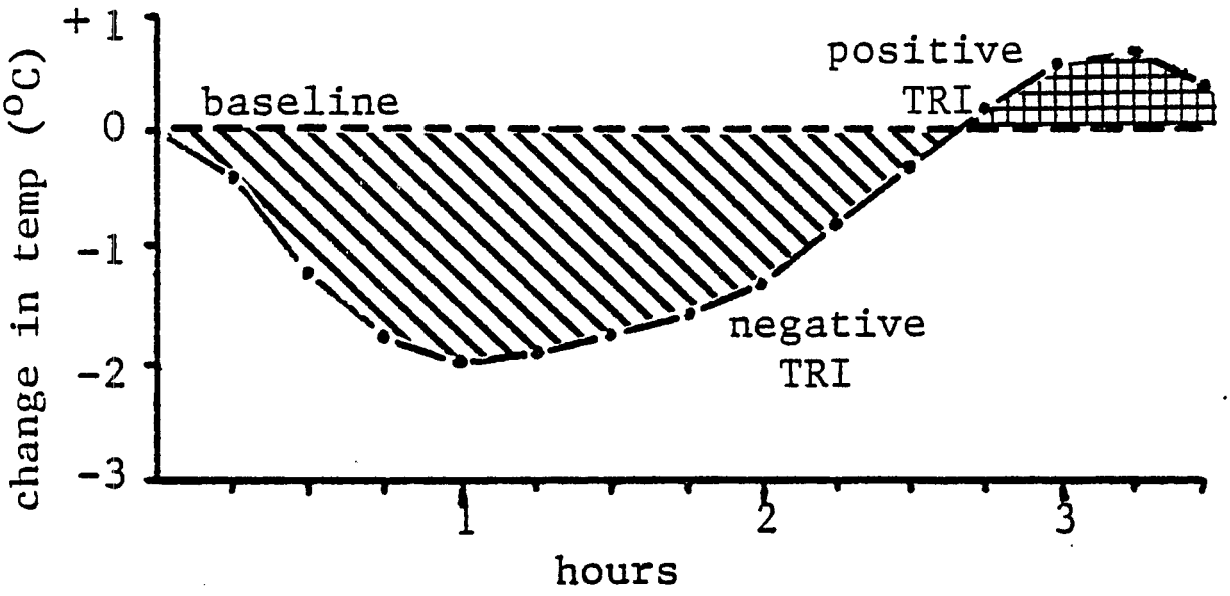

Figure 3. Thermal response index. TRI is a measurement of the area between the temperature curve and baseline. It is expressed as $^{o}C \cdot hr$; therefore a TRI of 1 is equivalent to a $1^{o}C$ change from baseline of one hour duration.

temperatures greater than baseline, were calculated for each rat. Mean TRIs for each age group were compared using analysis of variance.

Respiratory Depression

Rats were weighed, marked, placed in a polyfilm restraint cone (Cervical Dislocators, Wausau, WI) and allowed to adapt to restraint for 1 to 2 hours. Respiratory rate was measured in a specially designed 32 x 29 x 22 cm chamber which was manufactured by the author from a metal record cabinet (Figure 4). This chamber was lined with acoustic ceiling tile and had a built-in fan with a 50 mm diameter blade (Dayton 7C722) to provide air circulation and noise to mask laboratory sounds. Average respiratory rate for each five minute period was automatically computed by an Aim 65 microcomputer (Rockwell International, Anaheim, CA). The average respiratory rate for the 30 minute period prior to injection was defined as baseline. Rats were injected with morphine (i.v.) during light ether anesthesia. Respiratory rate was monitored for 2 to 4 hours after injection and results were expressed as maximum percent change from baseline.

A modified pneumograph (Sargent-Welch Scientific Co., Skokie IL) was connected via plastic tubing to a pressure transducer (P23Db, Stratham, Hato Rey, Puerto Rico) which was in turn connected to a chart recorder (Type R411 DYNOGRAPH, Beckman Instruments Inc., Schiller Park IL) with a Beckman type 9803 strain gauge coupler. Output from the recorder was amplified, half wave rectified, split

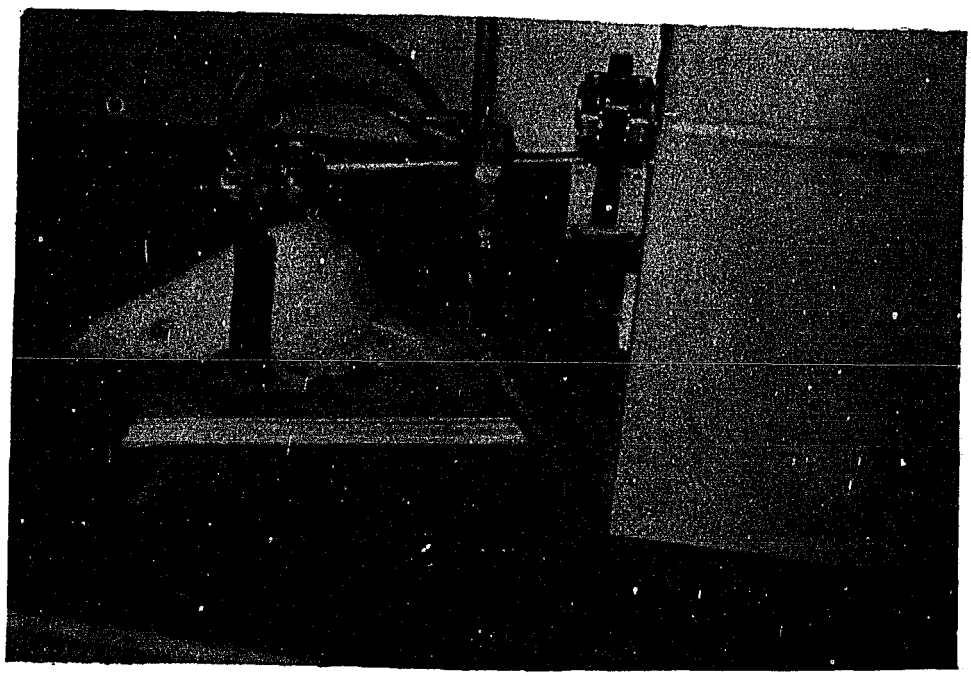

Figure 4. Rat in respiration measurement apparatus. Respiratory rates were measured in rats which were restrained in a plastic cone and placed in the sound attenuating chamber shown above on the right.

into 2 signals for comparison with reference levels, changed to a transistor-transistor logic (TTL) level and input to the Aim 65. The microcomputer timed 5 respiratory movements and calculated a respiratory rate if no non-respiratory movements were detected. If the new rate was within 30 per cent of the previous rate the new rate was saved for computation of an average rate for each 5 minute period.

Tolerance and Cross-tolerance

Drug tolerance and morphine tolerance were investigated in most experiments using the protocol shown in Figure 5. Each rat's thermic response to the second administration of a drug was measured as described above. Acute tolerance to a drug was determined 72 hours after the first dose, and the effect of morphine tolerance was determined 96 hours after the second. Morphine tolerance was produced by subcutaneous implantation of 75 mg morphine pellets prepared according to the method of Gibson and Tingstad (1970). Placebo pellets were prepared in the same manner, except without morphine. Pellets were implanted under light ether anesthesia, and the skin was closed with wound clips (Clay Adams, Parsippany NJ). Pellets were implanted 72 hours prior to drug testing and were left in place during the test. Rats which did not receive both doses were not used in group statistics for comparisons, therefore the mean thermic responses for the first injection in the tolerance comparisons varied slightly from the naive means depending on the

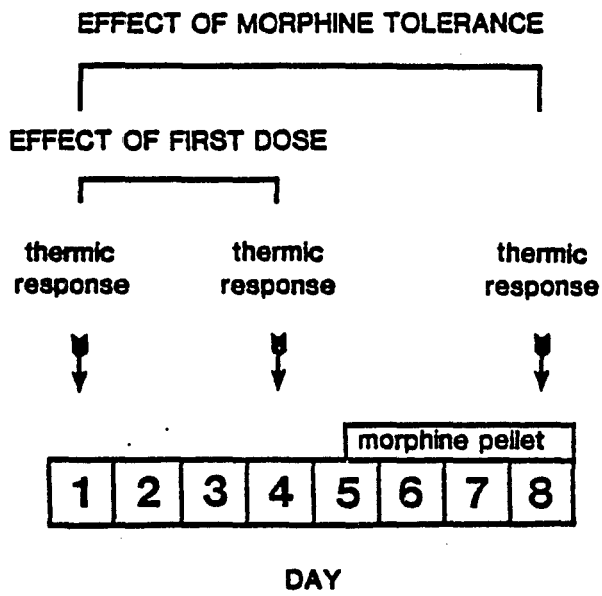

Figure 5. Drug tolerance protocol. Tolerance was assessed by administering a drug at three times (arrows). The effect of the first dose was determined by comparing the first and second doses. The effect of morphine tolerance or cross-tolerance was assessed by comparing the first and third doses.

number of rats which completed subsequent experiments. Differences in TRIs were determined using a t-test with an alpha level of .05.

Subcutaneous Absorption of Tritiated Water

The absorption of tritiated water (Ammersham, Arlington Heights IL) injected s.c. was studied to measure subcutaneous blood flow. Both the disappearance of tritiated water from the site of injection and the appearance of tritiated water in the blood were investigated. Tritiated water (15 uCi/ml) was injected subcutaneously below a previously marked and shaved 35 mm circle centered between the scapulae on the rat's back. At 3, 9, and 27 minutes after injection, groups of rats were sacrificed by decapitation and blood was collected into polypropylene tubes. Time points were chosen after a pilot experiment in 200 to 300 gram Sprague-Dawley rats allowed an estimate of time of absorption (Figure 6). The skin and underlying muscle bounded by the circle was excised with scissors, weighed and placed into screw cap tube for digestion. The tissue was digested in 10 ml 2N potassium hydroxide in methanol overnight at 40 ^{o}C. Radioactivity was measured in 0.2 ml of serum or digested in 5 ml Betaphase (West Coast Scientific, San Diego CA) after bleaching with 0.1 ml hydrogen peroxide and neutralizing with 0.1 ml concentrated acetic acid. The radioactivity injected into each rat was calculated after direct counts of the injection solution. Radioactivity in blood was expressed as DPM/ml blood, and

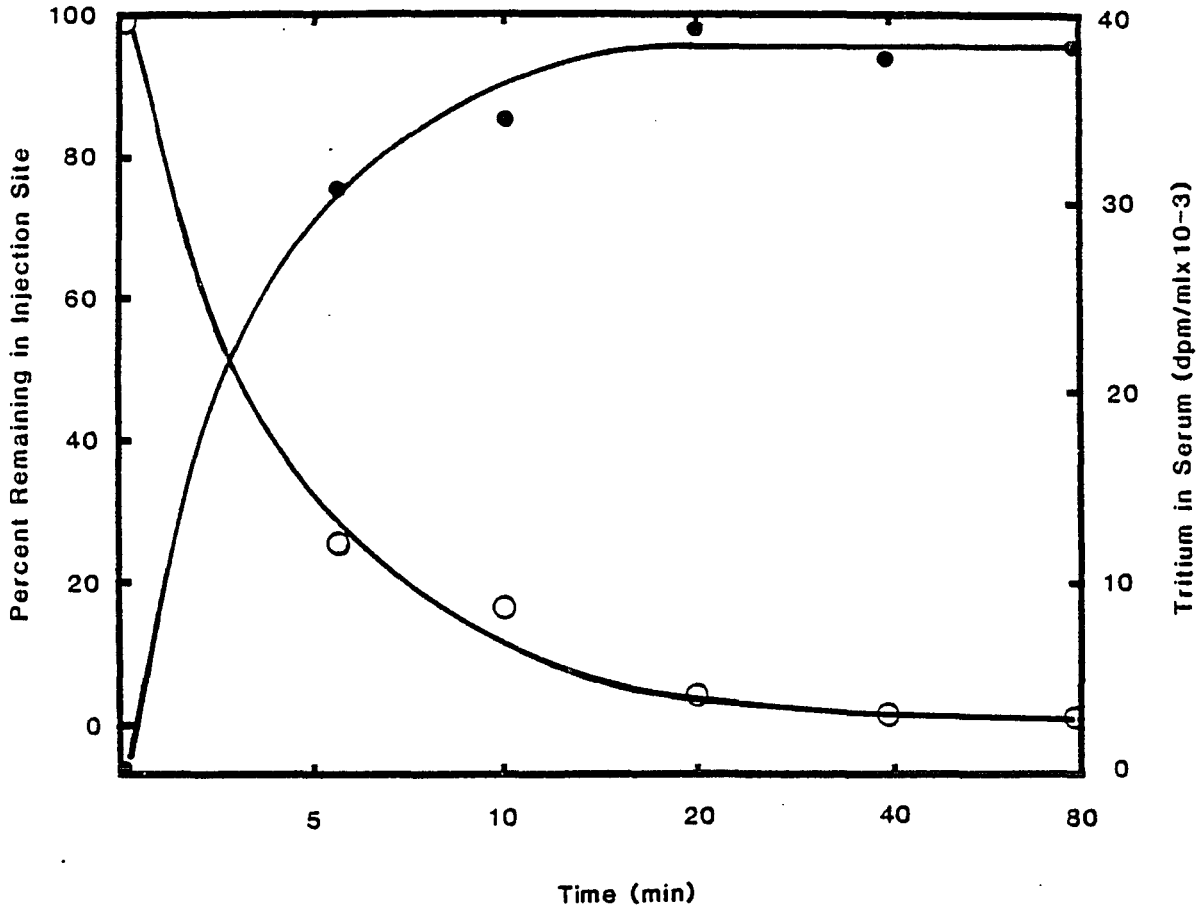

Figure 6. Subcutaneous absorption of tritiated water in Sprague-Dawley rats.

tritium remaining in the site was expressed as the percent of administered dose.

Analgesia

Measurement of analgesia was accomplished by the apparatus shown in Figure 7, which was designed to reproduce the tail-flick method of D'Armour and Smith (1941). Tail-flick, a spinally-mediated nociceptive reflex, was assessed by measuring time after activating a heat source until the rat abruptly moved his tail. Rats were placed in polyfilm restraint cones (Cervical Dislocators Inc., Wausau WI) for one hour prior to analgesia testing in order to minimize interference of stress with analgesia determinations. The rat tail was placed between guides 6 cm above a 500 watt projector bulb (CZA-CZB, Sylvania, Winchester KY) such that the light shined through a 1 cm diameter foil circle. The rat was placed so that the heat was aimed 8 cm posterior to the base of the tail. Latency was read from the built in timer (Time-it, Scientific Products, McGaw Park IL) which was automatically turned on at the same time as the bulb. Both the timer and the bulb were turned off manually by one remote switch when the rat moved his tail. In the absence of a response, the bulb was turned off at 12 seconds to avoid tissue damage. Results were expressed as percent increase in tail-flick latency above baseline (which was defined as the last 2 of 3 trials prior to drug injection). Drug responses were defined as the mean of two responses at the appropriate time after drug injection. The individual trials in both the baseline and drug response measurements were separated by

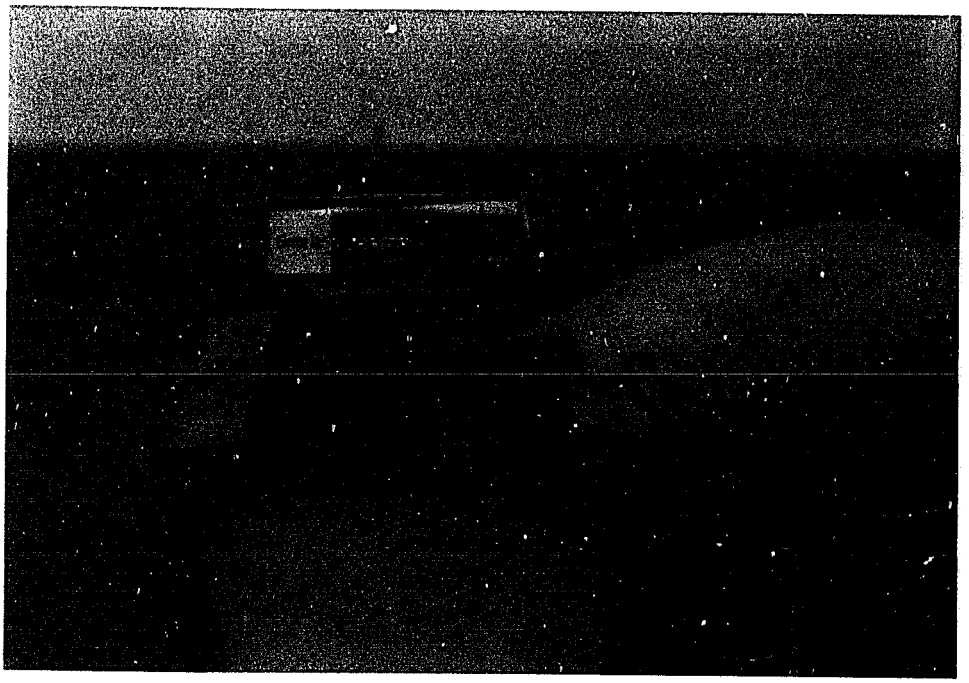

Figure 7. Rat on tail-flick apparatus. Latencies for the perception of heat on the tail of a rat were measured in this locally manufactured apparatus. A 500 watt projector bulb was the heat source. The timer was stopped manually from a remote switch when the rat moved his tail in response to heat.

2.2 minutes to allow the projector bulb to cool. Figure 8 shows that the increase in latency due to morphine is dose related in 200 to 300 grams Sprague-Dawley rats.

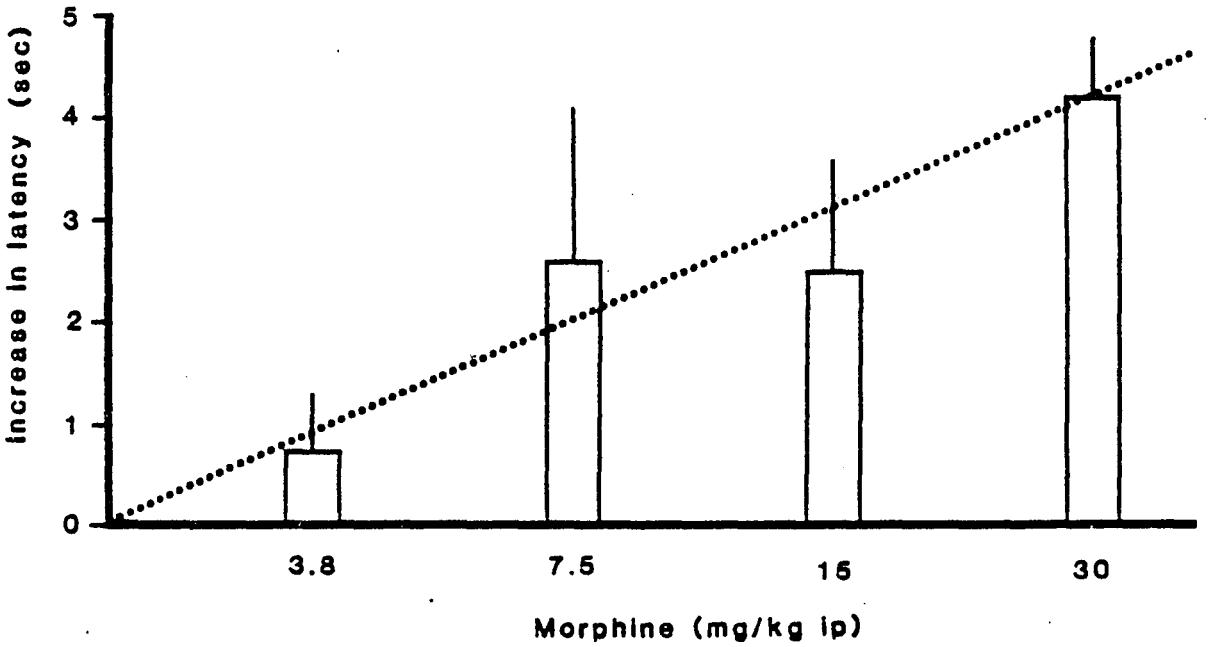

Figure 8. Analgesic response to morphine (i.p.) in Sprague-Dawley rats.

RESULTS

Aged Fischer 344 rats used in this study exhibited many of the characteristics of aging found in the aged human population. Most readily apparent were the superficial physical changes such as coarsening of the features and fur, visable lesions, curvature of the spine, muscular weakness or weight loss which occurred during the twilight of rat life.

Internal changes in senescent rats were also much more frequent than in the other groups. It was not the purpose of this study to characterize the pathological processes associated with aging, however, each autopsy of a senescent rat regardless of cause of death, elucidated gross visual pathologies such as hepatomegaly, splenomegaly, testicular tumors and lung or renal nodules.

Young rats were obviously smaller than the mature and senescent rats. Means of the of the rat group's mean weights during the initial experimental sessions were 273 ± 7, 347 ± 5 and 350 ± 6 grams for young, mature and senescent rats, respectively. Analysis of variance indicated that the weights were different ($F_{2,51}$= 57.6).

Once delivered to our animal facility, the death rate of senescent rats was much greater than the death rate of young or mature rats. Frequently some of the 26 to 28 month old rats died within a week after arrival, however, similar deaths in the 3 to 5 and 10 to 12 month old rats were unusual. The greater death rate was

probably due to age rather than treatment in the local animal facility. Senescent rats failed to survive initial treatments and i.c.v. cannula implantation as well as young or mature rats. Behavioral differences noted were a decrease in playful activities with other rats in the mature and senescent groups, and failure for some senescent rats to groom themselves.

Senescent rats thermoregulated at a lower rectal temperature than the young and mature rats. Means of the mean baselines rectal temperatures of the rat groups during all the initial experiments were 37.0 ± 0.1, 36.8 ± 0.1 and 36.0 ± 0.1 °C for the young, mature and senescent groups, respectively ($F_{2,51}$= 17.2).

Ambient Temperature Effects

Age-related differences in thermoregulation in the absence of drugs were evaluated by exposing rats to both a warm and cold environment. Rats were placed into the restrainer in the animal facilities which have an ambient temperature of 20 to 21 °C. After adjustment to restraint and measurement of baseline temperature, rats were moved into either a cold room (7.4 ± 0.4 °C) or a warm room (32.5 ± 0.1 °C) for thermic evaluation.

Cold Exposure

Acute exposure of the rats to a cold environment induced decreases in rectal temperature (Figure 9). Young and mature groups initially lost 2 to 3 °C from baseline and maintained the reduced body temperature for approximately 2.5 hours, after which additional

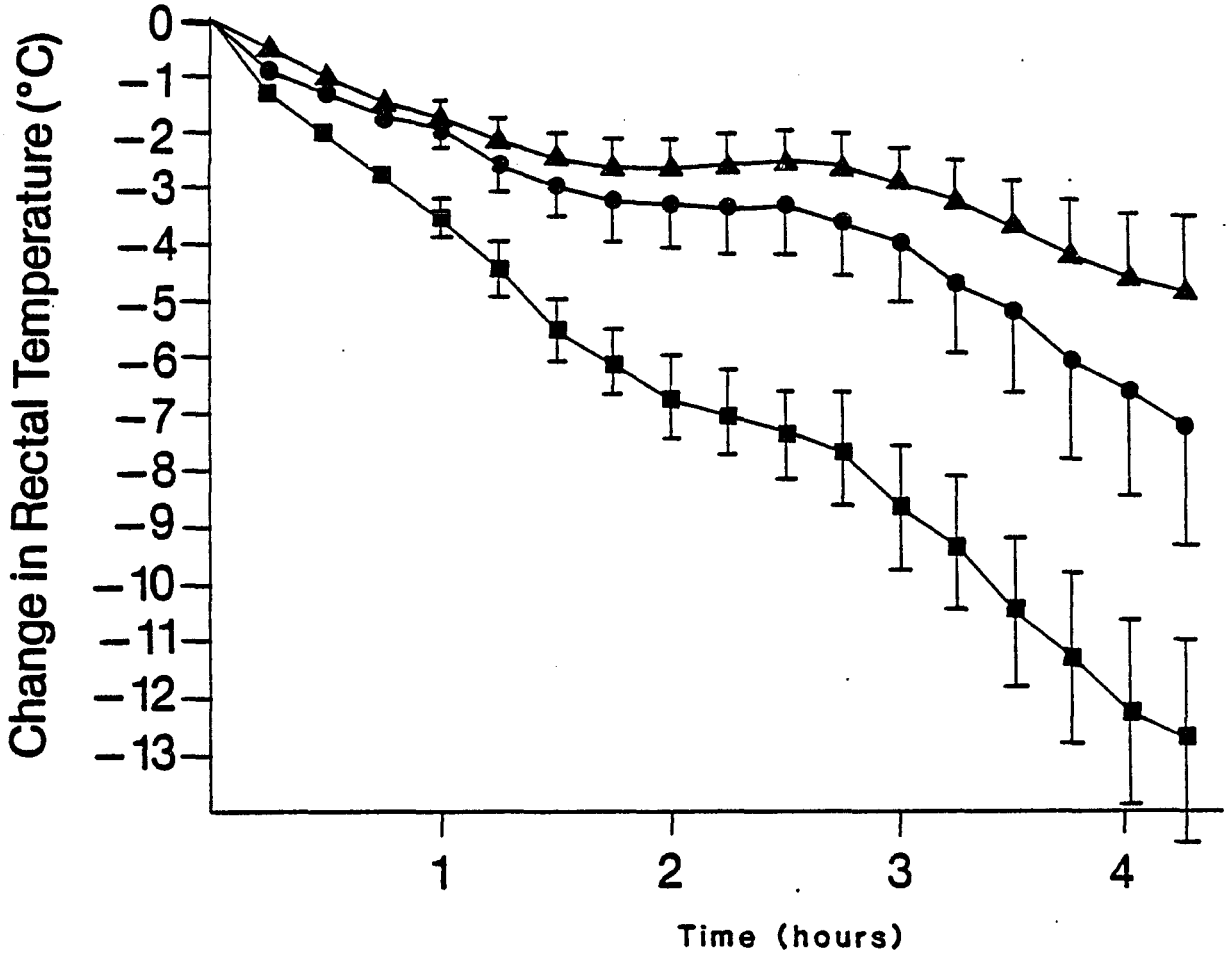

Figure 9. Thermic response to cold stress. Baseline temperatures were determined at an ambient temperature of 20 to 21 °C and then rats were moved into a cold room (7.6 ± 0.4 °C). Each point is the mean ± SE of changes from baseline rectal temperatures in 5 or 6 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

losses of 2 to 3 °C occurred. Senescent rats displayed an almost continuous loss of body heat for over 4 hours, by which time body temperature was diminished 12 °C. The cold stress experiment was terminated after 4.25 hours to avoid deaths due to hypothermia. During the period of exposure all rats lost 5 to 6 percent of their body weights. There were no differences in weight loss between groups. Table 1 summarizes the effects of a cold environment. The TRIs for the senescent rats were almost twice the TRIs for the young and mature rats. None of the rats had temperatures above baseline at any time point.

Warm Exposure

Acute exposure of the rats to a warm environment induced increases in rectal temperature (Figure 10). Temperatures rose continuously for the first hour in all groups and then slowly diminished in the young and mature rats. In the senescent rats, hyperthermia was maintained during the 5.75 hour period of measurement. All groups lost 5.1 to 5.6 percent of their body weight during the experiment. The rectal temperatures 5 hours after being exposed to the warm environment were different ($F_{2,14}= 3.9$, $P \leq .05$). The senescent rats were almost twice as hyperthermic as the young rats (1.7 vs 0.9 °C) at the 5 hour point. Table 2 summarizes the effects of the warm environment, and Table 3 summarizes changes in the thermoregulatory system with aging.

Table 1. Effects of an ambient temperature of 7.6 ± 0.4 °C on rectal temperature during a 4.25 hour exposure. Baseline temperatures were measured in the animal facility at an ambient temperature of 20 to 21 °C and then rats were moved into a cold room.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	6	314 ± 8	37.3 ± 0.4	-15.4 ± 3.9	- 7.2 ± 2.1	0.0 ± 0.0	0.0 ± 0.0
Mature	6	378 ± 10	36.9 ± 0.4	-11.5 ± 2.3	- 4.8 ± 1.3	0.0 ± 0.0	0.0 ± 0.0
Senescent	5	355 ± 8	36.4 ± 0.4	-27.0 ± 1.9	-11.4 ± 1.5	0.0 ± 0.0	0.0 ± 0.0
$F_{2,14}$=		13.6	7.0	7.2	3.7	-	-
Significance ($P \leq .05$)		*	*	*			

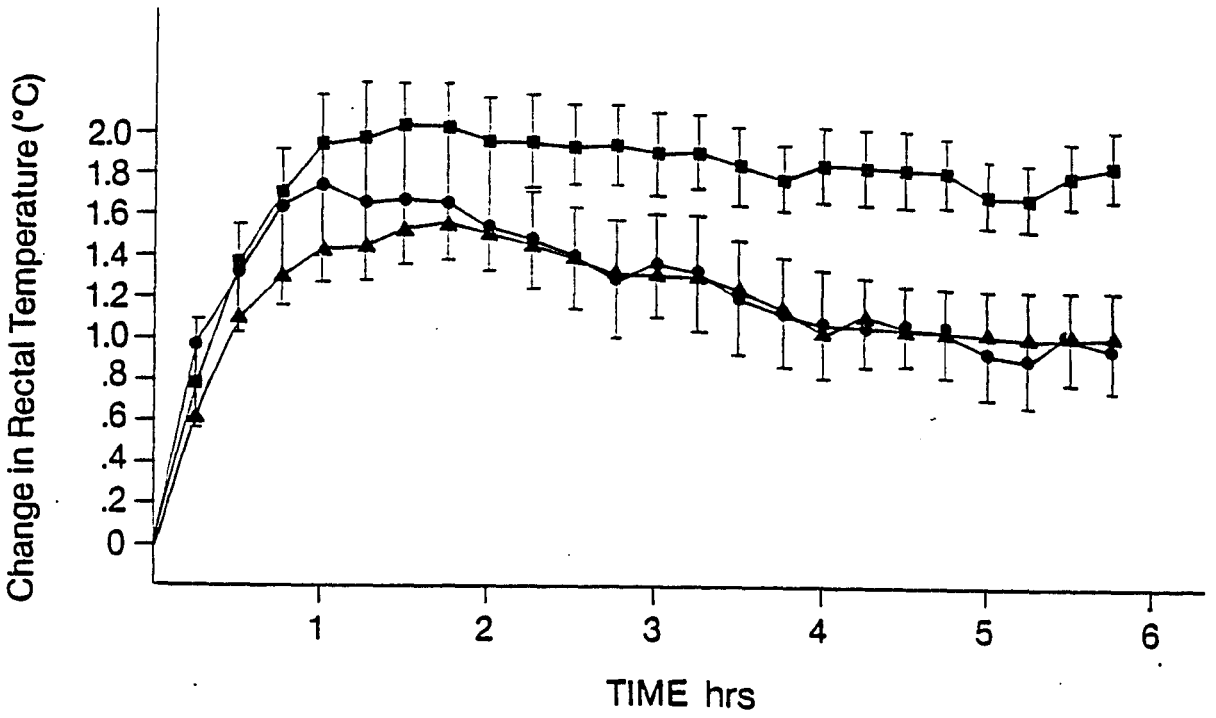

Figure 10. Response to heat stress. Baseline temperatures were determined at at ambient temperature of 20 to 21 °C, and then rats were moved into a warm room (32.5 ± 0.1 °C). Each point is the mean ± SE of changes from baseline rectal temperatures in 5 or 6 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 2. Effects of an ambient temperature of 32.5 ± 0.1 °C on rectal temperature during a 5.75 hour exposure. Baseline temperatures were measured in the animal facility at an ambient temperature of 20 to 21 °C, and then rats were moved into a warm room.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	6	302 ± 9	36.5 ± 0.3	- 0.0 ± 0.0	- 0.0 ± 0.0	7.4 ± 1.3	1.8 ± 0.2
Mature	6	357 ± 8	36.4 ± 0.2	- 0.0 ± 0.0	- 0.0 ± 0.0	7.0 ± 1.0	1.6 ± 0.2
Senescent	5	340 ± 12	36.0 ± 0.3	- 0.0 ± 0.0	- 0.0 ± 0.0	10.2 ± 1.1	2.1 ± 0.5
$F_{2,14}$=		7.9	0.7	-	-	2.1	1.3
Significance ($P \leq .05$)		*					

Table 3. Summary of changes in the thermoregulatory system with aging.

Parameter	Age-related difference	Response of senescent vs Young
Baseline	Yes	Decreased
Weight loss during restraint	No	-
Cold stess induced hypothermia	Yes	Increased
Warm stress induced hyperthermia	Yes	Increased

Morphine Effects

The effects of morphine on thermoregulation depended on dose and route of administration. Age-related differences in response to morphine were investigated after subcutaneous, oral, intracerebroventricular and intravenous administration.

Subcutaneous Administration

Morphine sulfate was injected in doses of 5, 25 & 125 mg/kg. Administration of a low dose of morphine (5 mg/kg) at an ambient temperature of 21.2 to 21.9 ^{o}C caused a transient hypothermia in all three age groups which was followed by a prolonged period of hyperthermia (Figure 11). The initial period of hypothermia showed no differences between the groups (Table 4). Rectal temperatures were a minimum of 0.5 to 1.0 ^{o}C below baseline between 15 and 30 minutes after injection. A rapid recovery from the hypothermia occurred in all groups until temperatures were a maximum of 0.6 to 1.2 ^{o}C at 2 to 3 hours. All groups lost 3.9 to 5.2 percent of body weight during the experiment. After the first 1.5 hours, the mature and senescent groups exhibited greater hyperthermia evidenced by both the integrated and maximum responses (Table 5). These groups exhibited approximately twice the maximum temperature increase from baseline and three times the integrated increase in temperature as the young group. After the peak hyperthermic response, temperatures in all groups began to fall at approximately the same rate.

A higher dose of morphine sulfate (25 mg/kg s.c.) caused hypothermia in all three age groups (Figure 12) when injected at an

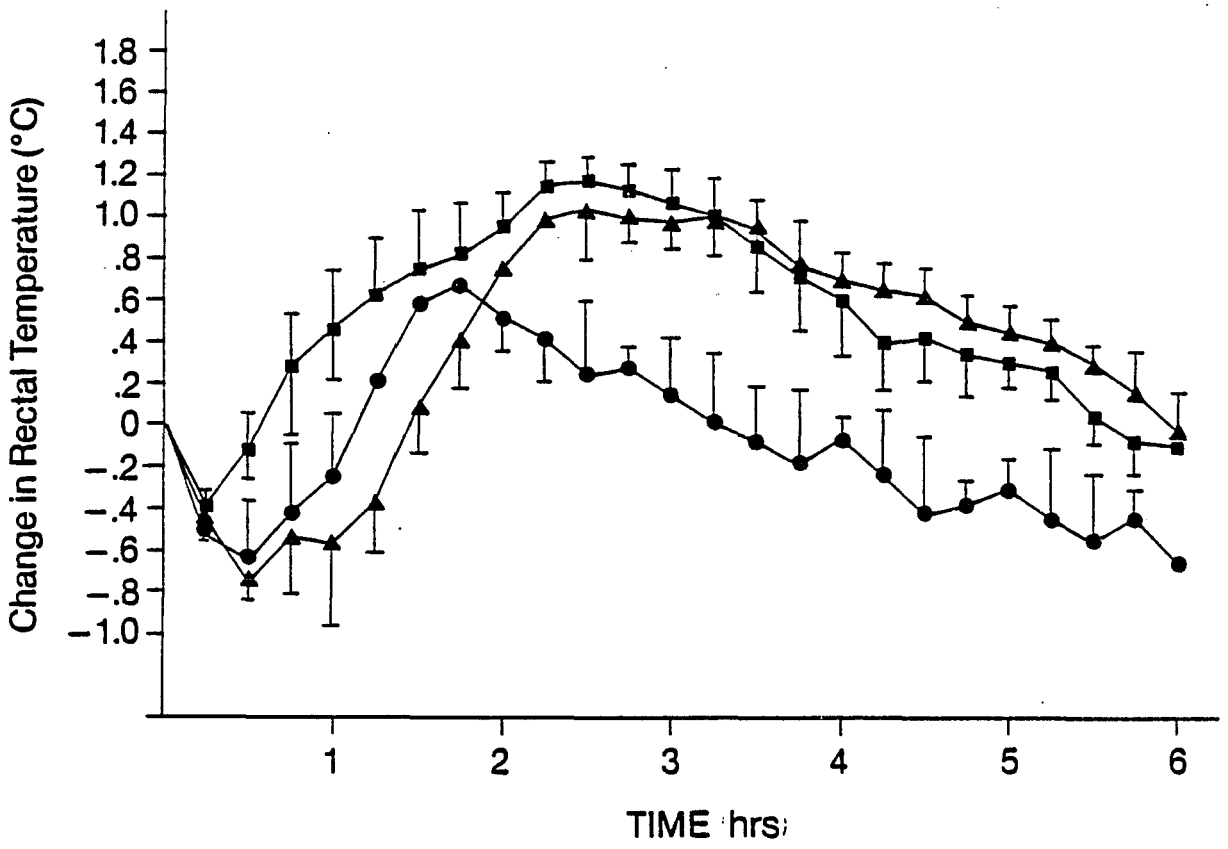

Figure 11. Thermic response to morphine (5 mg/kg s.c.). Each point is the mean ± SE of changes from baseline rectal temperatures in 4 or 5 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 4. Effects of morphine (5 mg/kg s.c.) on rectal temperature during the first 1.5 hours after injection.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	5	263 ± 2	36.3 ± 0.1	- 0.6 ± 0.2	- 0.8 ± 0.2	0.2 ± 0.1	0.6 ± 0.2
Mature	5	324 ± 20	36.0 ± 0.6	- 0.8 ± 0.2	- 1.0 ± 0.2	0.1 ± 0.1	0.3 ± 0.2
Senescent	4	350 ± 23	36.5 ± 0.1	- 0.2 ± 0.1	- 0.4 ± 0.1	0.6 ± 0.2	0.8 ± 0.3
$F_{2,11}$=		6.5	0.6	3.1	2.8	2.9	1.3
Significance (P≤.05)		*					

Table 5. Effects of morphine (5 mg/kg s.c.) on rectal temperature from 1.5 to 6.0 hours after injection. Same experiment as Table 3.

Group	N	Weight (g±SE)	Baseline (^{o}C±SE)	Neg TRI (^{o}C·hr±SE)	Max Hypo (^{o}C±SE)	Pos TRI (^{o}C·hr±SE)	Max Hyper (^{o}C±SE)
Young	5	263 ± 2	36.3 ± 0.1	- 1.0 ± 0.5	- 0.7 ± 0.2	0.8 ± 0.3	0.7 ± o.2
Mature	5	324 ± 20	36.0 ± 0.6	- 0.1 ± 0.1	- 0.3 ± 0.1	3.0 ± 0.3	1.2 ± 0.1
Senescent	4	350 ± 23	36.5 ± 0.1	- 0.1 ± 0.1	- 0.1 ± 0.1	2.9 ± 0.4	1.3 ± 0.1
$F_{2,11}$=		6.5	0.6	2.4	4.6	16.4	6.1
Significance ($P \leq .05$)		*			*	*	*

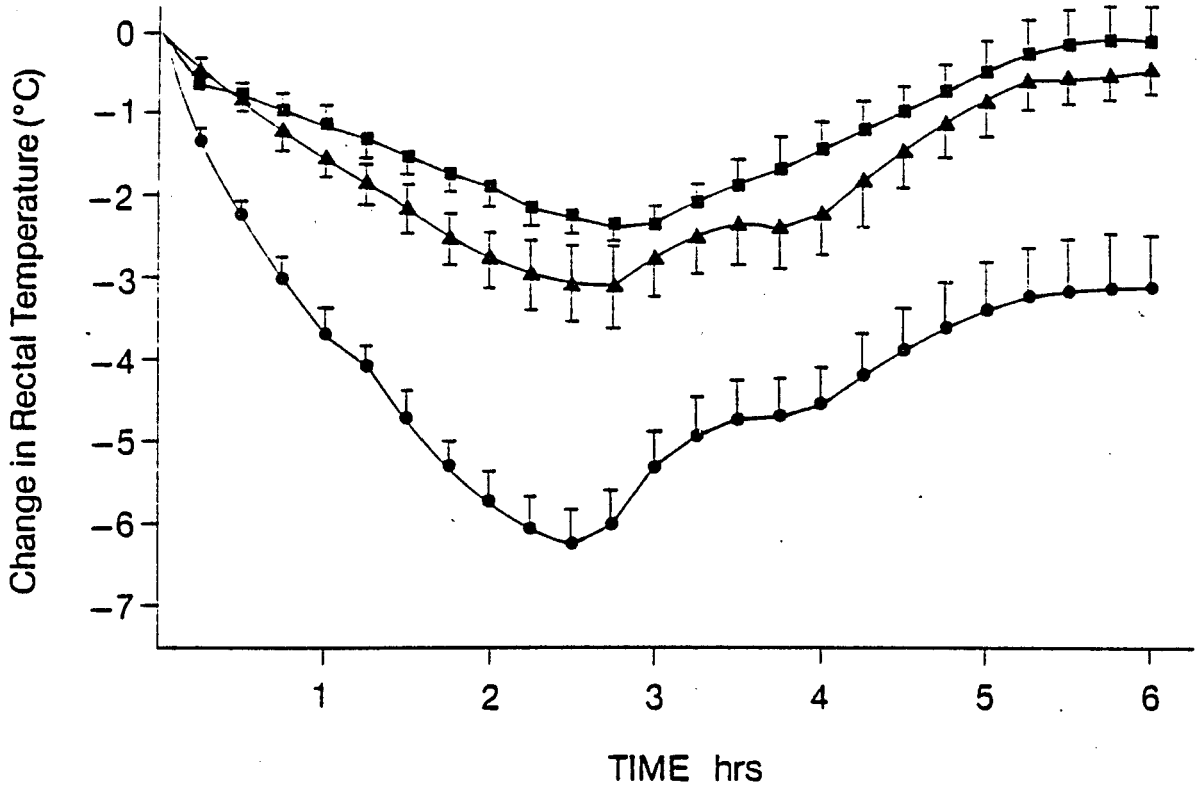

Figure 12. Thermic response to morphine (25 mg/kg s.c.). Each point is the mean ± SE of changes from baseline rectal temperatures in 6 or 7 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

ambient temperature of 21.7 to 22.1 °C. The fall in rectal temperature began immediately and reached a nadir at 2.5 to 3 hours. All groups were quiet for the first 2 to 2.5 hours after injection; then they became alert and sometimes agitated as evidenced by chewing on the restrainer. The young rats responded with more hypothermia than the mature and senescent groups (Table 6). Young rats exhibited at least twice the maximum response and twice the integrated response seen with mature and senescent rats. Rats lost 3.0 to 4.1 percent of their body weight during the experiment.

A very high dose of morphine (125 mg/kg s.c.) caused hypothermia and some deaths in all age groups when injected at an ambient temperature of 21.9 to 23.6 °C (Figure 13). In four of the senescent rats and one each of the mature and young rats, this dose was lethal, and data from these rats were not included in the thermic responses. Hypothermia of 2 to 4 °C which was produced in all groups was maximum at 1.5 to 2.0 hours. The mature and senescent groups had partially recovered by 4 hours, but the young group had not recovered baseline temperatures by 5.75 hours after injection. Table 7 summarizes the thermic effects of this dose.

In summary, subcutaneous morphine showed biphasic responses in all age groups. The predominantly hyperthermic response with 5 mg/kg became a hypothermic response at larger doses (25 and 125 mg/kg). The predominantly hyperthermic response shown in Figure 11 had a small initial hypothermic component. With larger doses this initial hypothermic response was of greater magnitude and duration; it became

Table 6. Effects of morphine (25 mg/kg s.c.) on rectal temperature during 6 hours after injection.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	7	269 ± 13	36.0 ± 0.3	-25.1 ± 1.8	- 6.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
Mature	6	382 ± 10	36.2 ± 0.3	-10.8 ± 1.8	- 3.2 ± 0.5	0.3 ± 0.3	0.3 ± 0.2
Senescent	6	388 ± 15	35.6 ± 0.1	- 7.9 ± 1.3	- 2.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.3
$F_{2,16}$=		27.1	1.6	32.1	30.0	1.5	2.2
Significance ($P \leq .05$)		*		*	*		

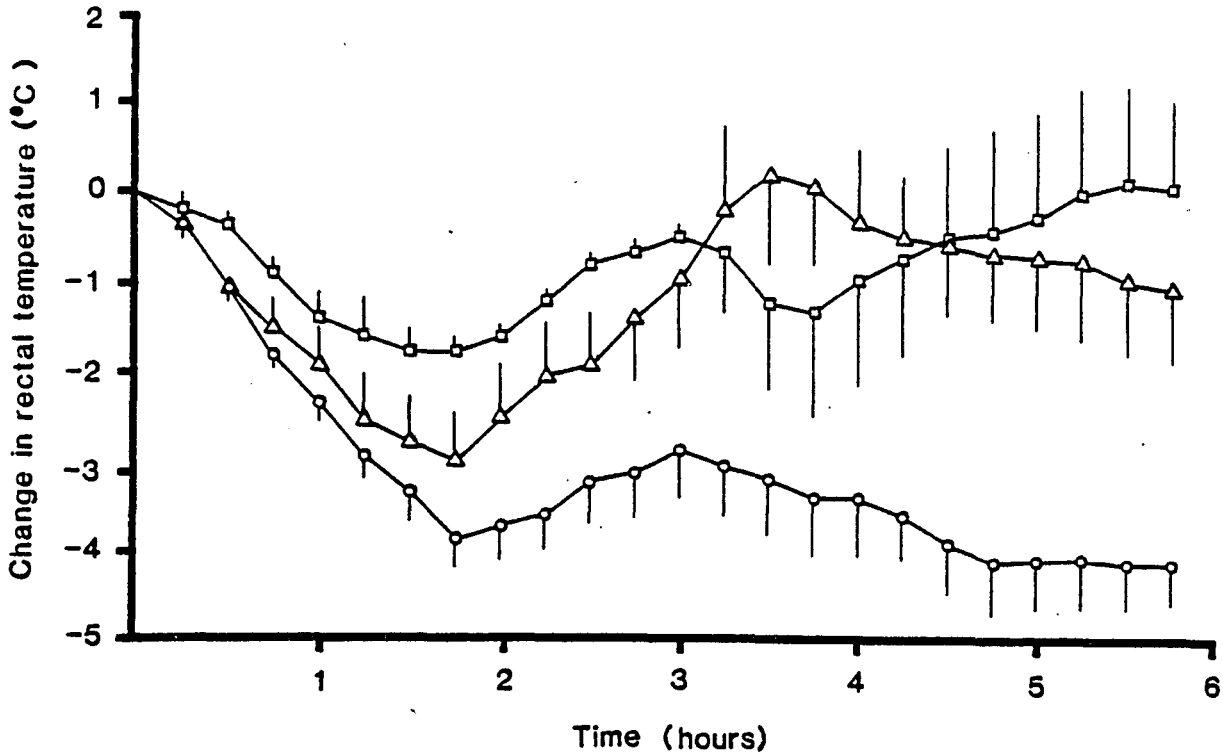

Figure 13. Thermic response to morphine (125 mg/kg s.c.). Each point is the mean ± SE of changes from baseline rectal temperatures in 2 to 6 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 7. Effects of morphine (125 mg/kg s.c.) on rectal temperature during 5.75 hours after injection. Weight and baseline data include the 4 senescent, 1 mature and 1 young rats that did not survive the treatment.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	6	287 ± 6	37.4 ± 0.2	-18.2 ± 2.0	- 4.3 ± 0.5	0.2 ± 0.2	0.0 ± 0.0
Mature	5	338 ± 25	37.0 ± 0.2	- 9.8 ± 4.2	- 3.0 ± 0.4	1.7 ± 1.3	1.0 ± 0.7
Senescent	2	380 ± 11	36.7 ± 0.1	- 5.6 ± 2.3	- 2.1 ± 0.3	1.0 ± 1.0	1.1 ± 0.8
$F_{2,10}$=		10.6	3.2	3.4	2.4	0.9	1.4
Significance ($P \leq .05$)		*					

the predominant response. The hyperthermic response which was predominant at the low dose was usually still present as an "overshoot" or recovery hyperthermia. Subcutaneous morphine was dramatically more hypothermic in the young rats than it was in the mature and sensecent rats (Figure 14). If present, age-related differences in hyperthermia caused by subcutaneous morphine are not dramatic (Figure 15).

Oral Administration

Morphine sulfate was administered by gastric intubation in fasted rats of 25, 50 and 100 mg/kg. Administration of the lowest oral dose of morphine (25 mg/kg) at an ambient temperature of 22.4 to 22.9 ^{o}C caused a predominantly hyperthermic response in all groups (Figure 16). The hyperthermic response in the young group was immediate and the peak hyperthermia of 1.5 ^{o}C above baseline occurred at approximately 2 hours after administration. Mature and senescent groups had a delay of approximately 1 hour before hyperthermia of equivalent magnitude resulted. Table 8 shows that the hyperthermic responses during the 6 hour period of measurement were not different. However, the hyperthermic TRIs calculated for the first 2 hours were 2.2 ± 0.3, 0.9 ± 0.2 and 0.7 ± 0.2 ^{o}C·hr for young, mature and senescent groups respectively ($F_{2,16}$= 11.2). These calculations indicate that the young rats responded more quickly, but to the same extent, as the mature and senescent rats did.

Doubling the dose of oral morphine to 50 mg/kg resulted in a similar response (Figure 17). With this dose, the more rapid onset

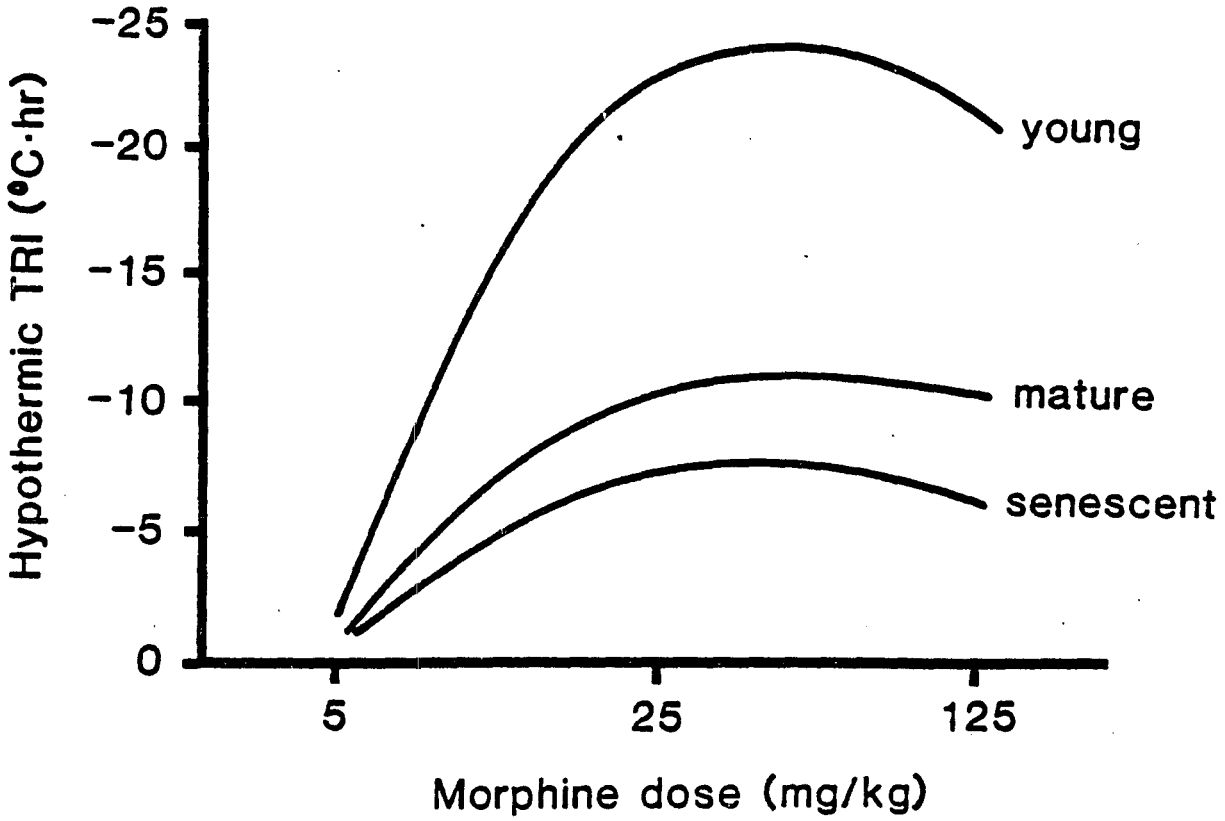

Figure 14. Hypothermic response to subcutaneous morphine. Each dose represents the mean TRI in 2 to 7 rats.

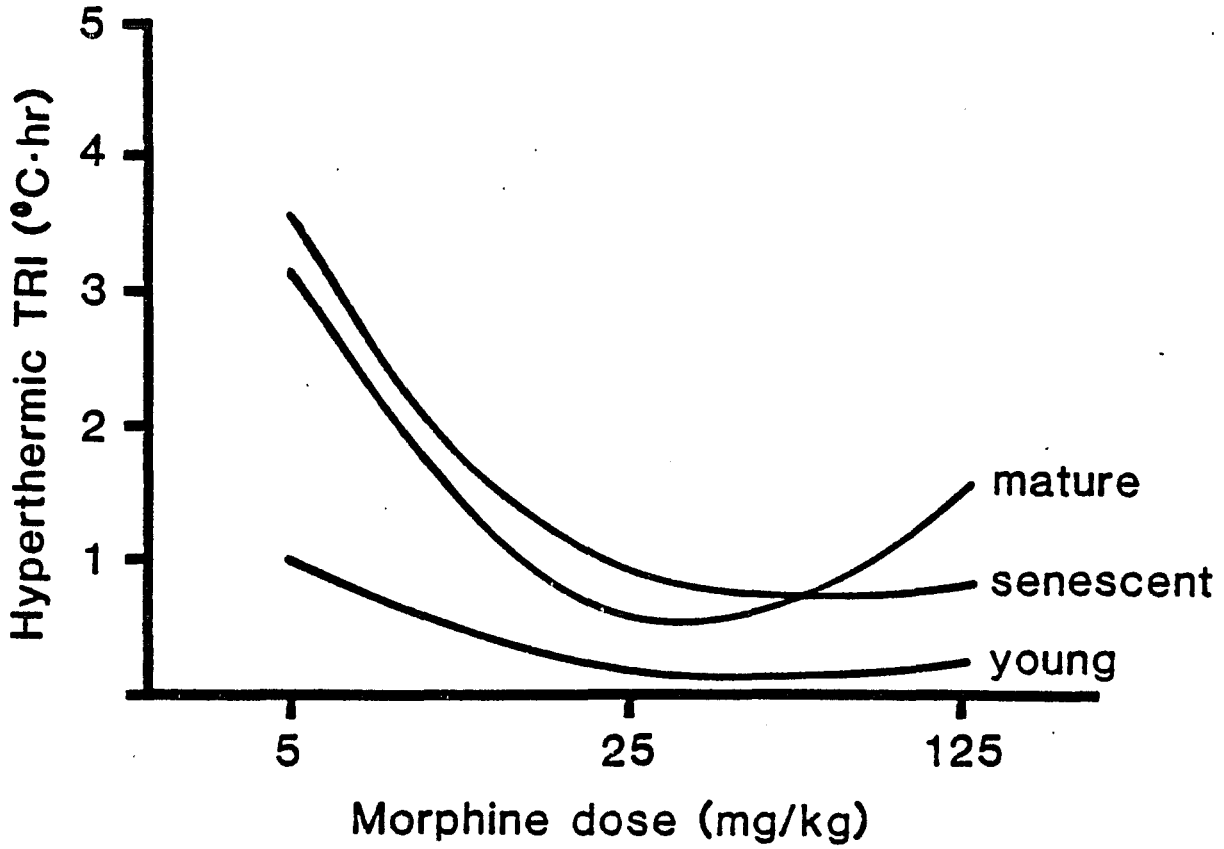

Figure 15. Hyperthermic response to subcutaneous morphine. Each dose represents the mean TRI in 2 to 7 rats.

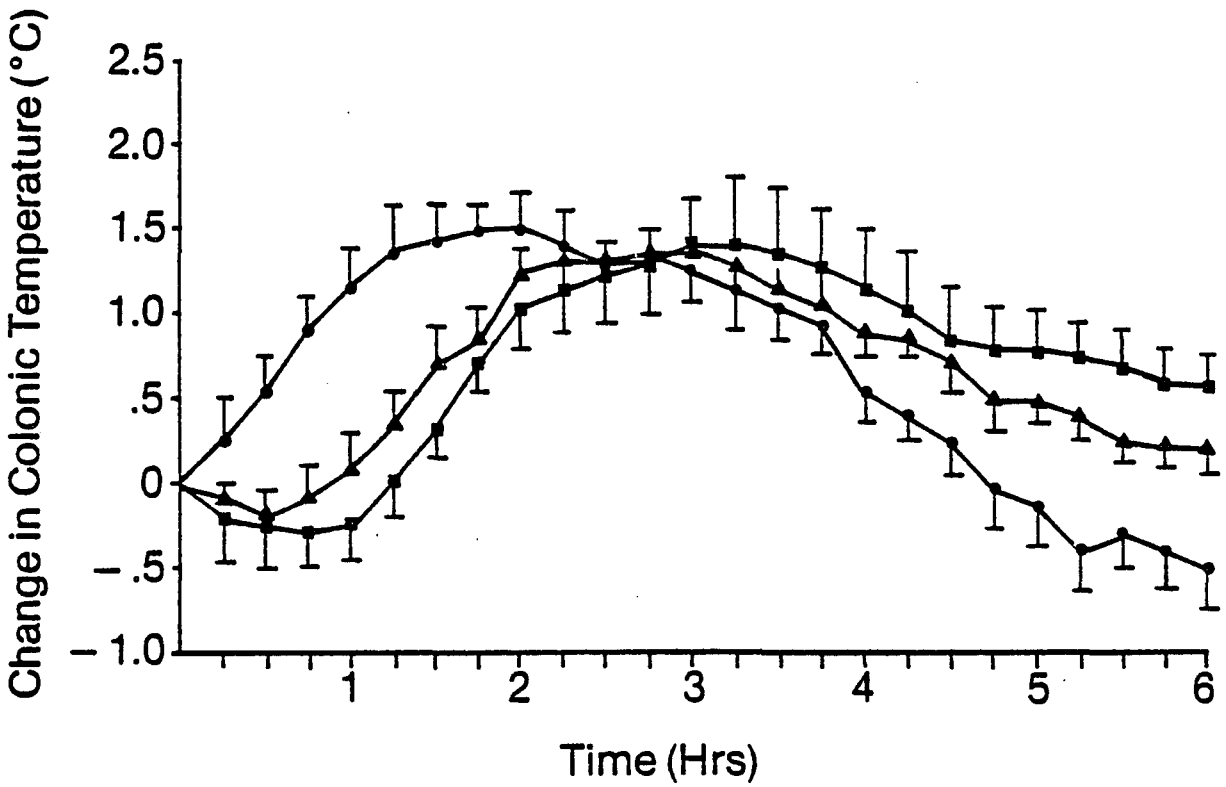

Figure 16. Thermic response to morphine (25 mg/kg p.o.). Each point is the mean ± SE of changes from baseline rectal temperatures in 6 or 7 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 8. Effects of morphine (25 mg/kg p.o.) on rectal temperature during 6 hours after intubation.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	6	204 ± 3	36.8 ± 0.2	- 0.7 ± 0.2	- 0.6 ± 0.2	4.7 ± 0.7	1.6 ± 0.1
Mature	6	336 ± 3	37.2 ± 0.1	- 0.2 ± 0.1	- 0.3 ± 0.1	4.3 ± 0.2	1.5 ± 0.1
Senescent	7	358 ± 8	36.7 ± 0.1	- 0.5 ± 0.1	- 0.4 ± 0.1	4.8 ± 1.2	1.6 ± 0.4
$F_{2,16}$=		20.8	2.2	2.0	1.7	0.1	0.1
Significance ($P \leq .05$)		*					

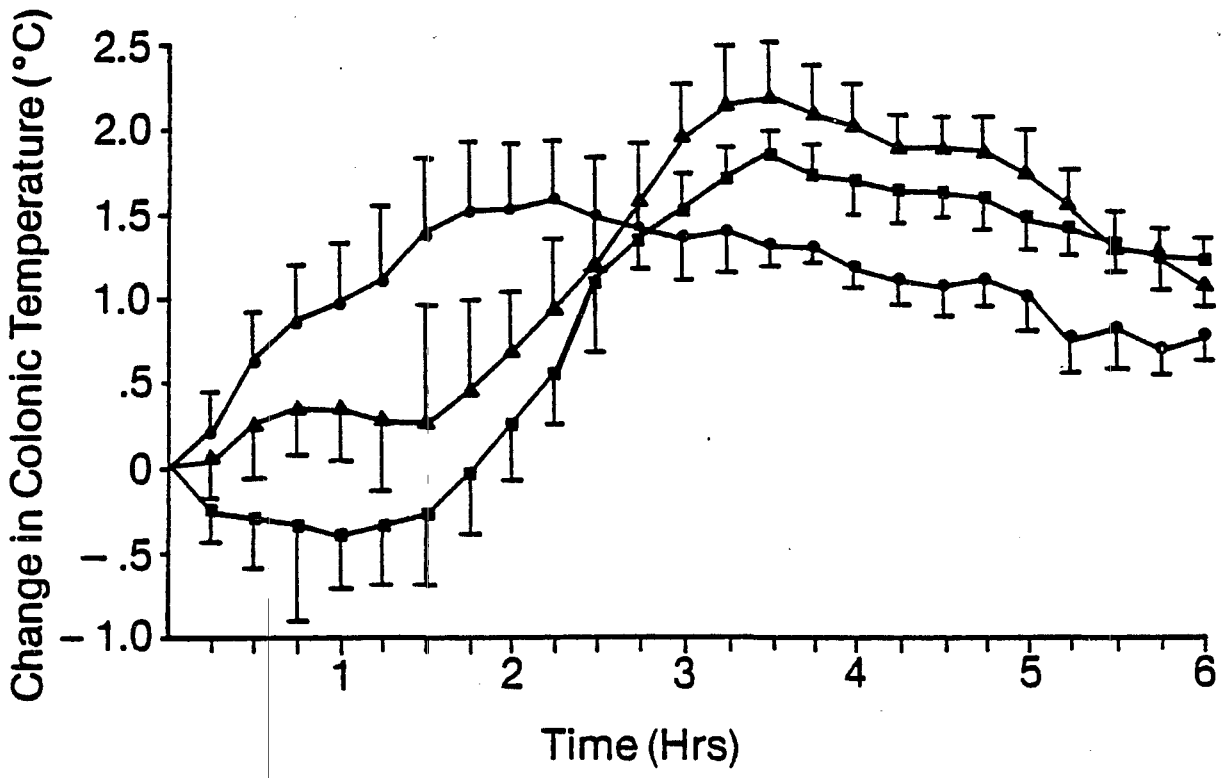

Figure 17. Thermic response to morphine (50 mg/kg p.o.). Each point is the mean ± SE of changes from baseline rectal temperatures in 6 or 7 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

of hyperthermia in the young group was again apparent, however, the integrated responses over the whole measurement period were not different (Table 9).

When 100 mg/kg was intubated, the hyperthermic response of rats of all ages was delayed for 2 hours (Figure 18). Neither the integrated nor maximum responses showed any age-related differences (Table 10).

Intracerebroventricular Administration

Morphine was administered into the left lateral ventricle (i.c.v.) in doses of 50, 100, 150 and 200 micrograms. Injection of 50 ug of morphine at an ambient temperature of 21.1 to 22.0 ^{o}C resulted in very small changes in rectal temperatures (Figure 19). Table 11 summarizes the thermic data. A larger dose of morphine (100 ug i.c.v.) resulted in slight hypothermia in all groups (Figure 20). Rectal temperature reached a minimum between 2 and 3 hours post injection and recovered to baseline by 4 hours. Hypothermia was equivalent in all groups (Table 12).

A large dose of morphine (150 ug i.c.v.) caused hypothermia in all groups at an ambient temperature of 20.3 to 21.6 ^{o}C (Figure 21). The young and mature groups responded after 1 hour with hypothermia of 1.5 to 2.5 ^{o}C and reached the nadir at 3 hours. In contrast, the senescent group responded almost immediately with a gradual decrease in rectal temperature during the 4.25 hour period of measurement. The senescent rats had greater hypothermia during the measurement period (Table 13). The minimum temperatures did not differ between

Table 9. Effects of morphine (50 mg/kg p.o.) on rectal temperature during 6 hours after intubation.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	7	231 ± 11	37.3 ± 0.1	- 0.3 ± 0.2	- 0.3 ± 0.2	6.9 ± 0.8	2.0 ± 0.1
Mature	6	323 ± 10	36.8 ± 0.2	- 0.4 ± 0.4	- 0.5 ± 0.5	8.0 ± 0.8	2.4 ± 0.2
Senescent	7	326 ± 14	36.6 ± 0.1	- 0.9 ± 0.4	- 0.7 ± 0.3	6.3 ± 0.5	2.1 ± 0.1
$F_{2,17}$=		19.9	6.8	1.3	0.6	1.4	1.8
Significance ($P \leq .05$)		*	*				

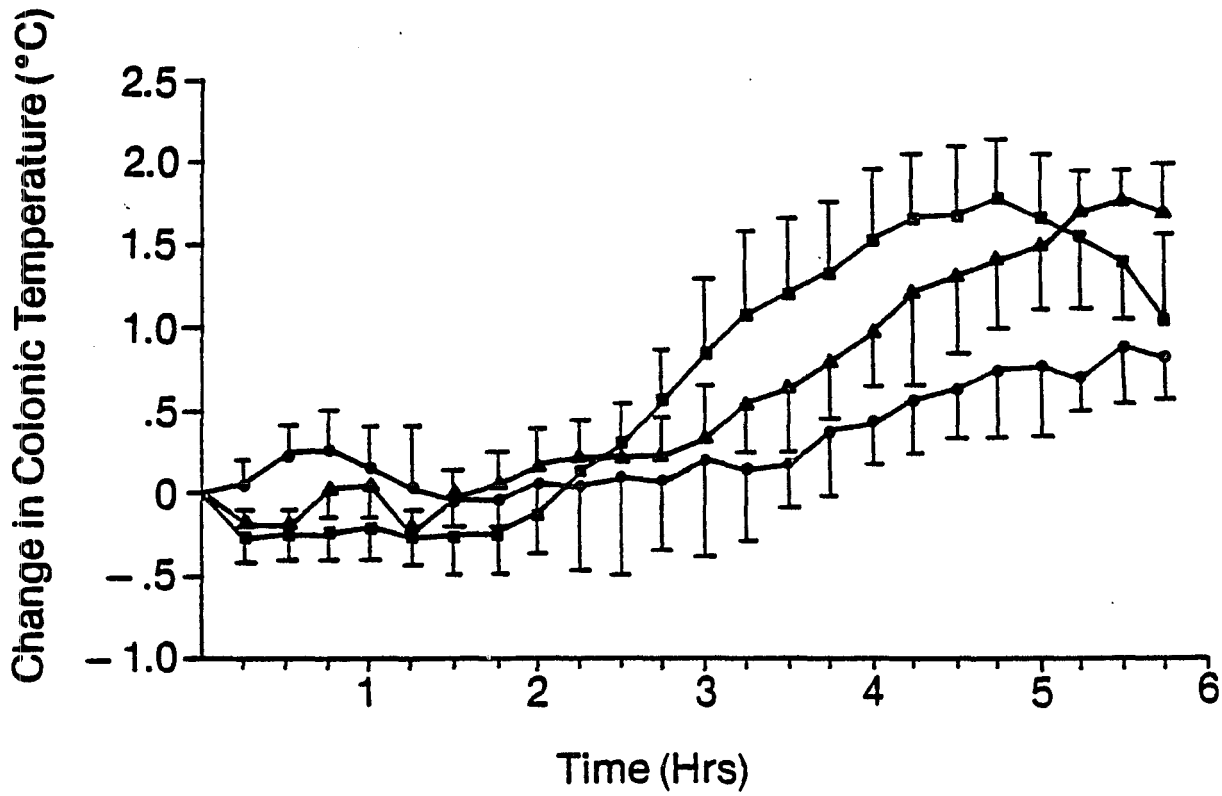

Figure 18. Thermic response to morphine (100 mg/kg p.o.). Each point is the mean ± SE of changes from baseline rectal temperatures in 5 or 6 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 10. Effects of morphine (100 mg/kg p.o.) on rectal temperature during 5.75 hours after intubation.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	6	278 ± 11	37.3 ± 0.2	- 0.8 ± 0.5	- 0.8 ± 0.4	4.2 ± 1.0	1.2 ± 0.2
Mature	6	347 ± 14	36.7 ± 0.1	- 0.7 ± 0.4	- 0.6 ± 0.2	3.8 ± 0.8	1.8 ± 0.2
Senescent	5	329 ± 14	36.2 ± 0.5	- 0.7 ± 0.2	- 0.5 ± 0.1	4.7 ± 1.4	1.9 ± 0.4
$F_{2,15}$=		7.4	3.7	0.1	0.2	0.2	3.6
Significance ($P \leq .05$)		*	*				

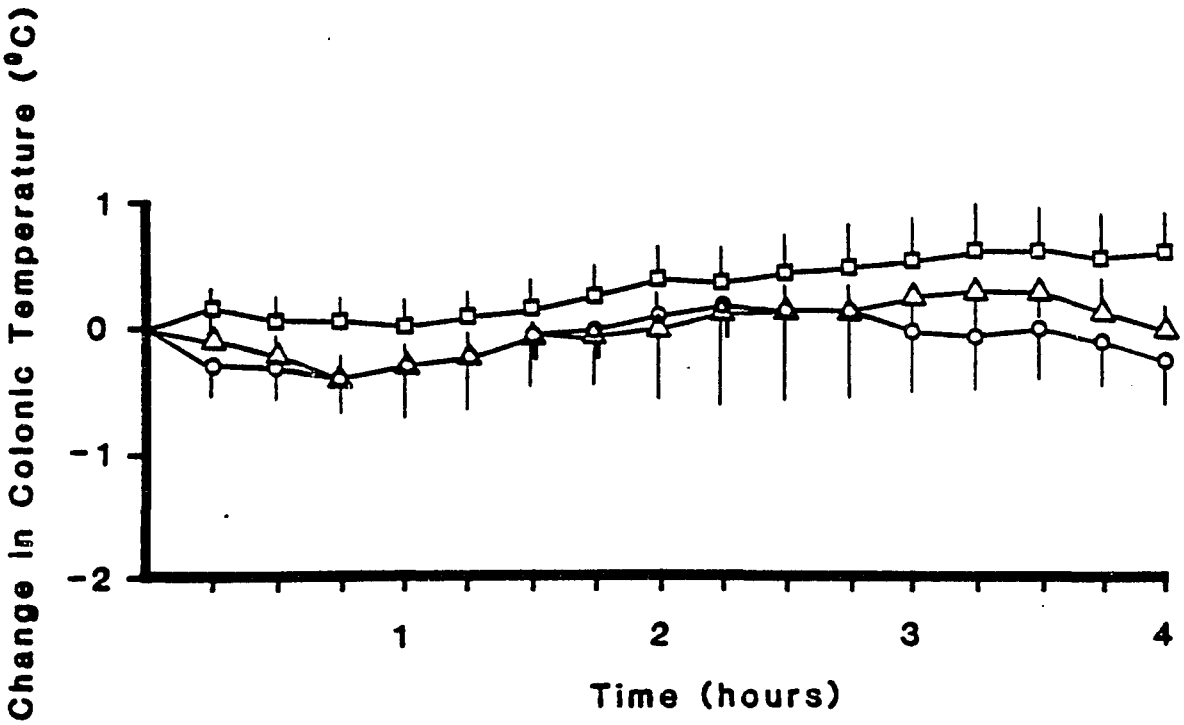

Figure 19. Thermic response to morphine (50 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 5 or 6 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 11. Effects of morphine (50 ug i.c.v.) on rectal temperature during 4 hours after injection.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	6	273 ± 5	37.8 ± 0.1	- 1.7 ± 0.9	- 0.8 ± 0.3	1.3 ± 0.7	0.7 ± 0.3
Mature	5	356 ± 9	37.4 ± 0.1	- 1.1 ± 0.5	- 0.4 ± 0.1	0.6 ± 0.4	0.4 ± 0.2
Senescent	5	351 ± 8	35.9 ± 0.3	- 0.3 ± 0.2	- 0.2 ± 0.1	1.7 ± 0.8	0.7 ± 0.3
$F_{2,13}$=		40.9	27.4	1.2	2.3	0.7	0.4
Significance ($P \leq .05$)		*	*				

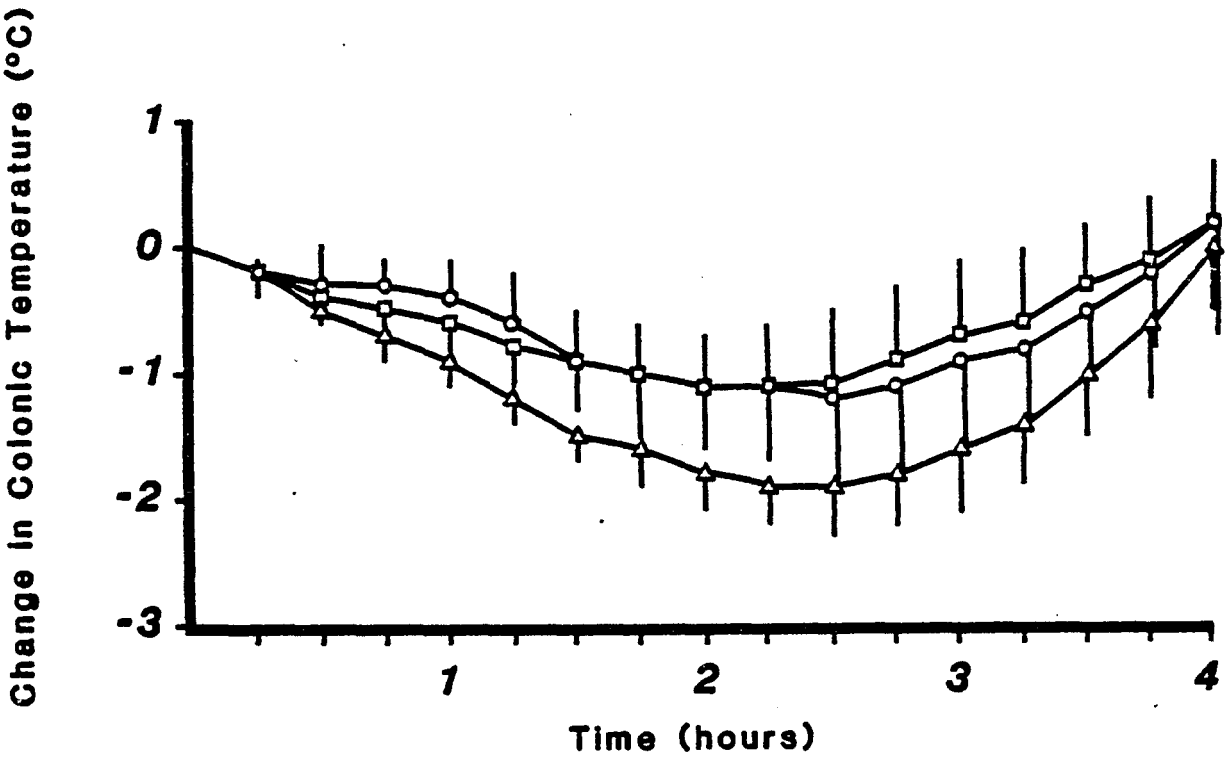

Figure 20. Thermic response to morphine (100 ug i.c.v.) Each point is the mean ± SE of changes from baseline rectal temperatures in 7 to 9 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 12. Effects of morphine (100 ug i.c.v.) on rectal temperature during 4 hours after injection.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	7	274 ± 6	37.7 ± 0.2	- 3.7 ± 1.4	- 1.6 ± 0.5	1.3 ± 0.6	1.1 ± 0.3
Mature	9	339 ± 8	37.3 ± 0.1	- 5.1 ± 1.1	- 2.1 ± 0.4	0.6 ± 0.2	0.8 ± 0.3
Senescent	9	377 ± 9	36.5 ± 0.2	- 3.5 ± 1.3	- 1.4 ± 0.5	1.2 ± 0.6	0.8 ± 0.3
$F_{2,24}$=		46.0	9.9	0.5	0.7	0.5	0.3
Significance ($P \leq .05$)		*	*				

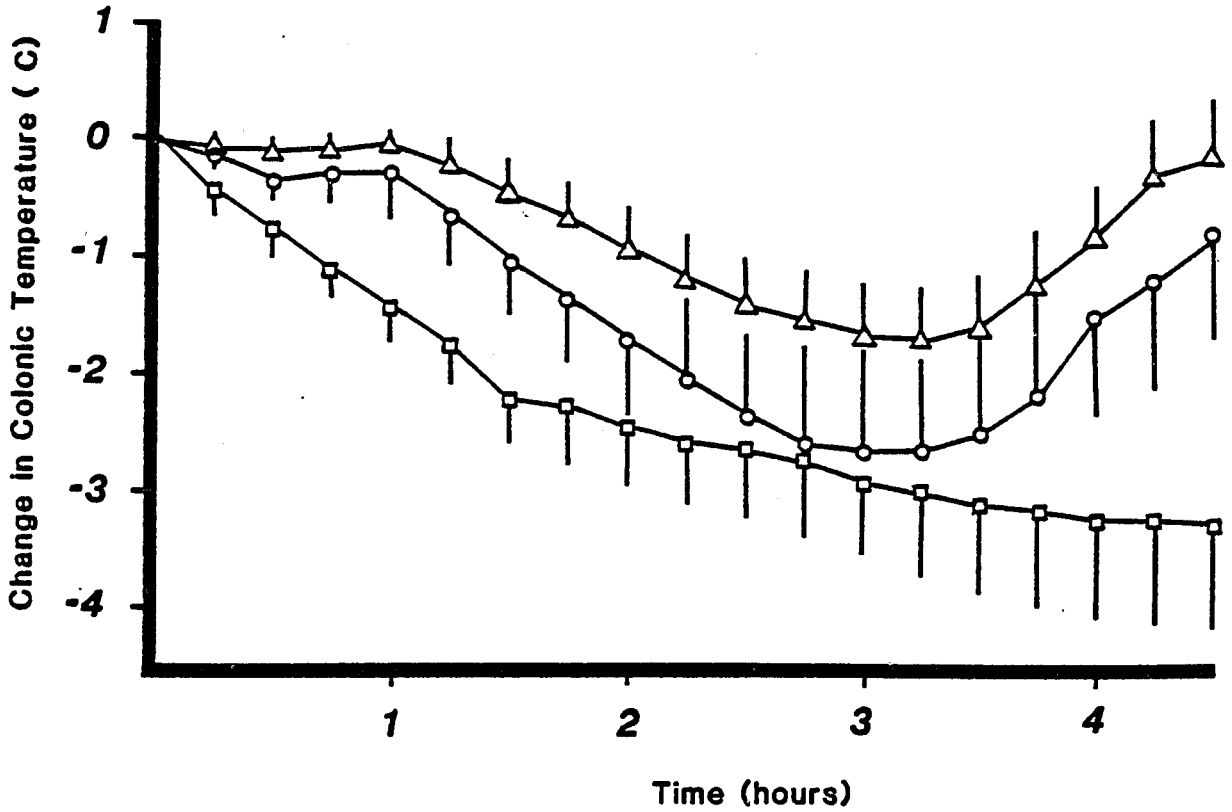

Figure 21. Thermic response to morphine (150 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 7 to 10 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 13. Effects of morphine (150 ug i.c.v.) on rectal temperature during 4.5 hours after injection.

Group	N	Weight (g±SE)	Baseline (^{o}C±SE)	Neg TRI (^{o}C·hr±SE)	Max Hypo (^{o}C±SE)	Pos TRI (^{o}C·hr±SE)	Max Hyper (^{o}C±SE)
Young	8	272 ± 3	37.7 ± 0.1	- 7.6 ± 2.3	- 3.1 ± 0.7	0.9 ± 0.6	0.7 ± 0.3
Mature	10	318 ± 12	37.1 ± 0.1	- 4.4 ± 1.1	- 2.0 ± 0.4	0.8 ± 0.4	0.7 ± 0.3
Senescent	7	354 ± 6	36.1 ± 0.4	-12.7 ± 1.3	- 3.8 ± 0.7	0.5 ± 0.5	0.3 ± 0.2
$F_{2,22}$=		29.9	10.8	6.5	2.5	0.2	1.1
Significance (P≤.05)		*	*	*			

the groups, but the senescent rat's temperatures did not return to basal during the experiment. This dose caused vocalization and occasionally shaking of the whole body and tail.

A very large dose of morphine (200 ug i.c.v.) caused hypothermia in all groups at an ambient temperature of 20.7 to 21.7 ^{o}C (Figure 22). Four rats in the young group and one in the mature group died during the measurement period or the subsequent 24 hours. The behavioral response to this dose was very striking in all age groups. Approximately 10 minutes after i.c.v. injections the rats became violent. They strained against the restraint and vocalized rhythmically with bulging eyes. Several rats convulsed. Rats became quiet 20 to 30 minutes after the injection but were excited again by touch or the vocalizations of an adjacent rat. There were no clear differences in thermic response (Table 14).

In summary, the injection of morphine, i.c.v., caused hypothermia which reached a nadir at 2.5 to 4 hours after administration, depending on the dose. Morphine administered directly into the ventricle did not cause maximum hypothermia as rapidly as morphine administered subcutaneously. In contrast to the age-related differences with subcutaneous morphine, i.c.v. morphine caused the greatest hyperthermia in the senescent group, but only at the highest doses which also caused vocalization and violent behavior (Figure 23). Intracerebroventricular morphine did not cause a significant measure of hyperthermia.

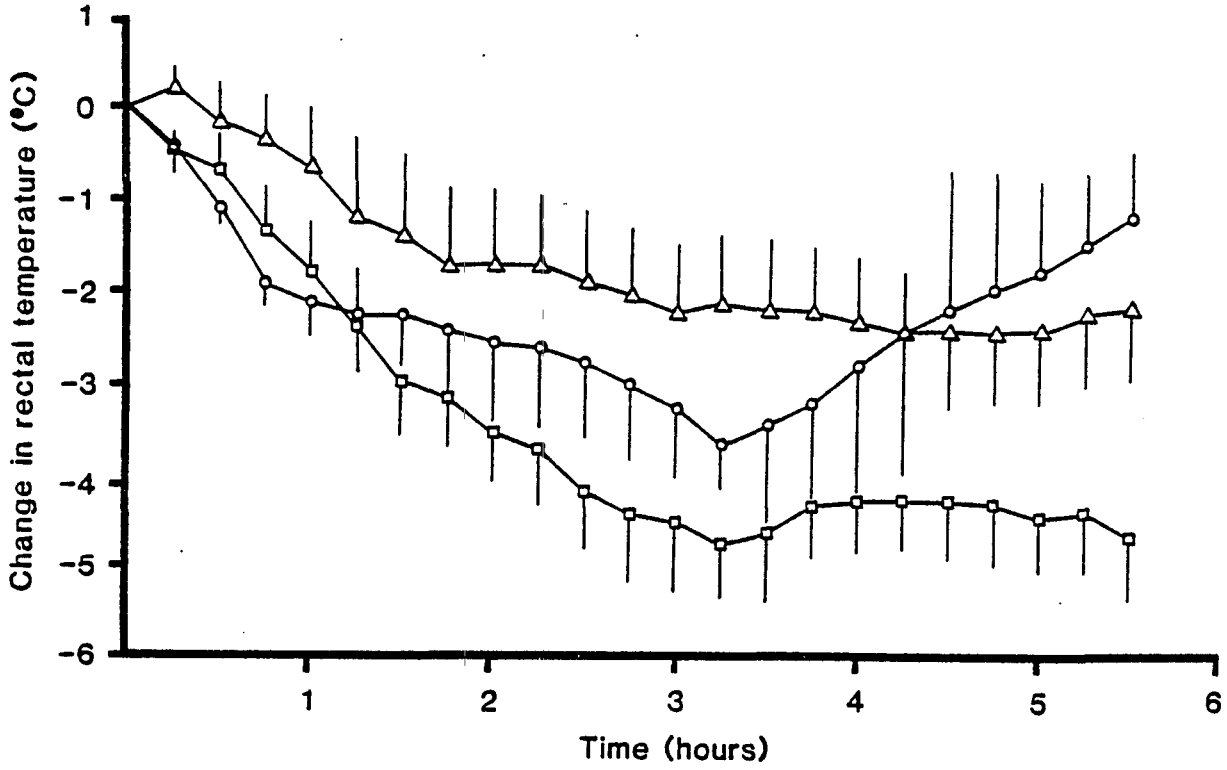

Figure 22. Thermic response to morphine (200 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 3 to 9 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 14. Effects of morphine (200 ug i.c.v.) on rectal temperature during 5.5 hours after injection. Weight and baseline data include 4 young and 1 mature rats that did not survive treatment.

Group	N	Weight (g±SE)	Baseline (^{o}C±SE)	Neg TRI (^{o}C·hr±SE)	Max Hypo (^{o}C±SE)	Pos TRI (^{o}C·hr±SE)	Max Hyper (^{o}C±SE)
Young	3	277 ± 7	36.9 ± 0.3	-12.9 ± 4.5	- 3.7 ± 0.9	0.3 ± 0.3	0.3 ± 0.3
Mature	9	345 ± 12	36.5 ± 0.2	-11.2 ± 3.1	- 3.2 ± 0.8	3.8 ± 2.1	0.8 ± 0.4
Senescent	6	371 ± 9	36.1 ± 0.2	-19.6 ± 3.0	- 5.4 ± 0.6	0.1 ± 0.1	0.1 ± 0.1
$F_{2,16}$=		24.3	2.1	1.8	2.1	1.5	2.0
Significance (P≤.05)		*					

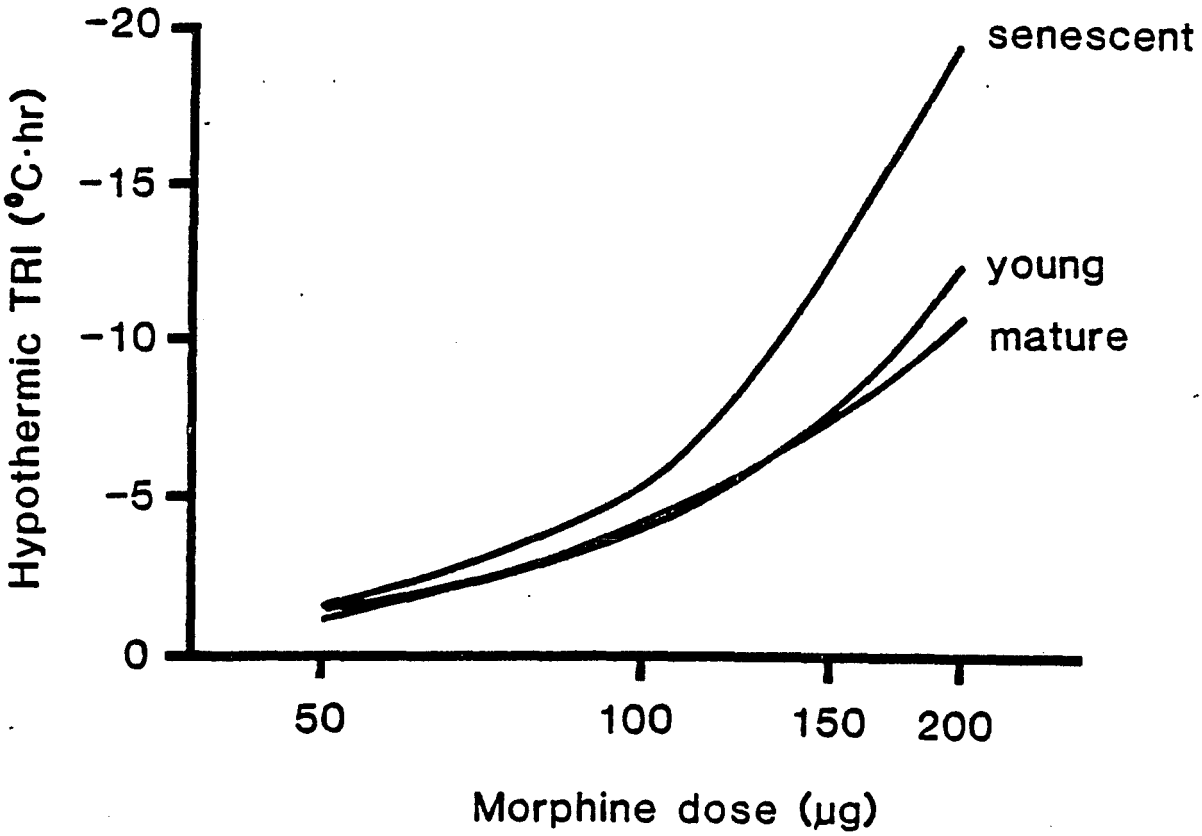

Figure 23. Hypothermic response to i.c.v. morphine. Each dose represents the mean TRI in 3 to 9 rats.

Intravenous Administration

Morphine injected intravenously (25 mg/kg) at an ambient temperature of 21.3 to 21.7 °C caused a hypothermic response in all groups (Figure 24). Rectal temperatures dropped 1.5 to 2 °C in the mature and senescent groups and 3 °C in the young group. All groups recovered basal temperatures by 4 hours. The maximum hypothermic response of the young rats was greater than the other rats (Table 15). Table 16 summarizes the thermic effects of morphine.

Ethanol Effects

The effects of ethanol on rectal temperature were investigated using two intraperitoneally administered doses. A low dose of ethanol (1.5 g/kg) caused hypothermia of 1.5 to 2 °C which was long lasting at an ambient temperature of 21.8 to 22.7 °C (Figure 25). No differences in thermic response were present (Table 17).

A large dose of ethanol (3 g/kg i.p.) caused a pronounced hypothermia of 7 °C in all groups when administered at an ambient temperature of 21.9 to 22.8 °C (Figure 26). No differences in thermic response were present (Table 18).

Transmitter Effects

Thermic effects of i.c.v. administered neurotransmitters were compared using acetylcholine, dopamine and norepinephrine. Doses of each transmitter which would produce a hypothermia of 1 to 2 °C were chosen for investigation.

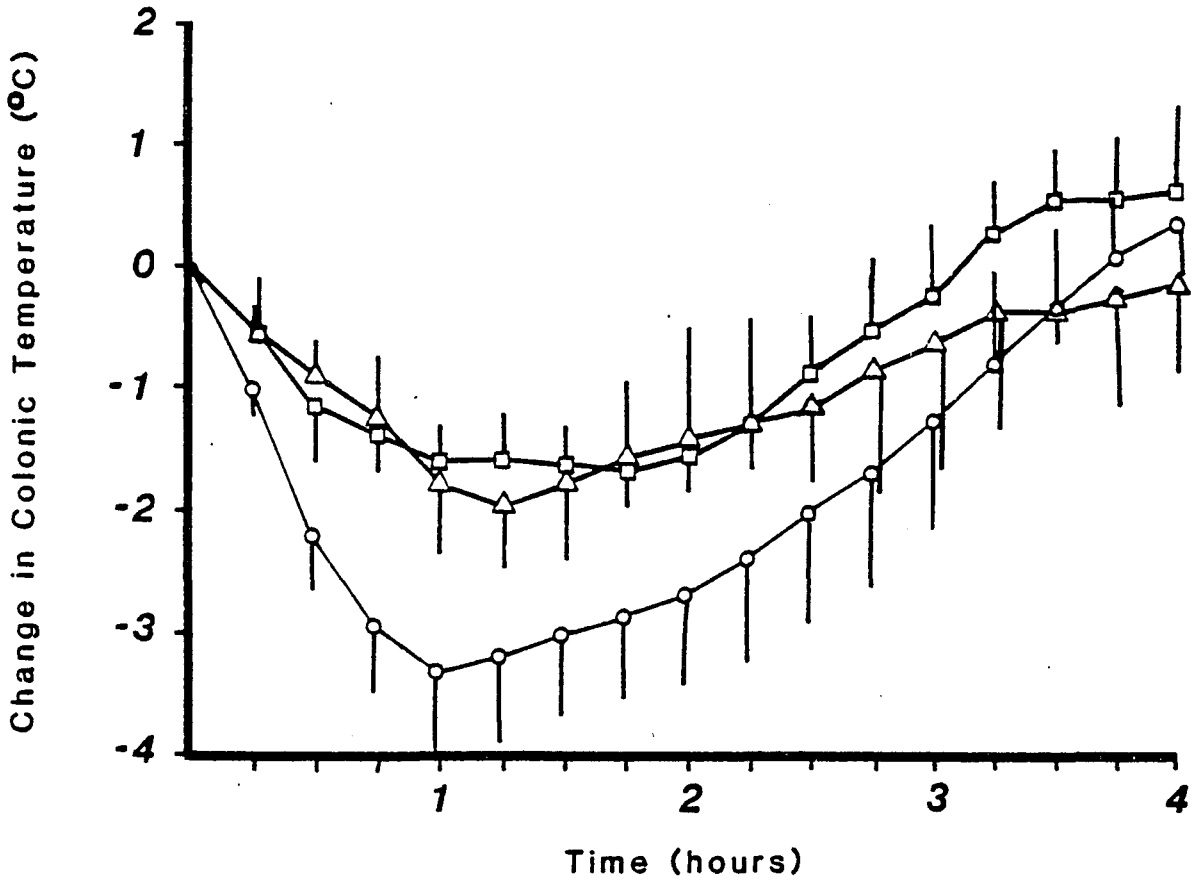

Figure 24. Thermic response to morphine (25 mg/kg i.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 4 or 6 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 15. Effects of morphine (25 mg/kg i.v.) on rectal temperature during 4 hours after injection.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	6	288 ± 14	36.8 ± 0.1	- 8.2 ± 2.1	- 3.9 ± 0.7	0.8 ± 0.4	0.7 ± 0.3
Mature	4	345 ± 15	36.6 ± 0.3	- 5.1 ± 2.0	- 2.3 ± 0.4	1.0 ± 0.8	0.9 ± 0.4
Senescent	6	344 ± 15	34.5 ± 1.0	- 4.4 ± 1.2	- 2.1 ± 0.3	1.6 ± 0.6	1.6 ± 0.4
$F_{2,13}$=		5.2	3.7	1.4	4.2	0.5	2.2
Significance ($P \leq .05$)		*			*		

Table 16. Summary of the thermic effects of morphine.

Parameter	Age-related Difference	Response of Senescent vs Young
Morphine hypothermia (subcutaneous)	Yes	Decreased
Morphine hyperthermia (subcutaneous)	Questionable	Increased
Morphine hyperthermia (oral)	No	-
Morphine hypothermia (i.c.v.)	Yes	Increased
Morphine hypothermia (i.v.)	No	-

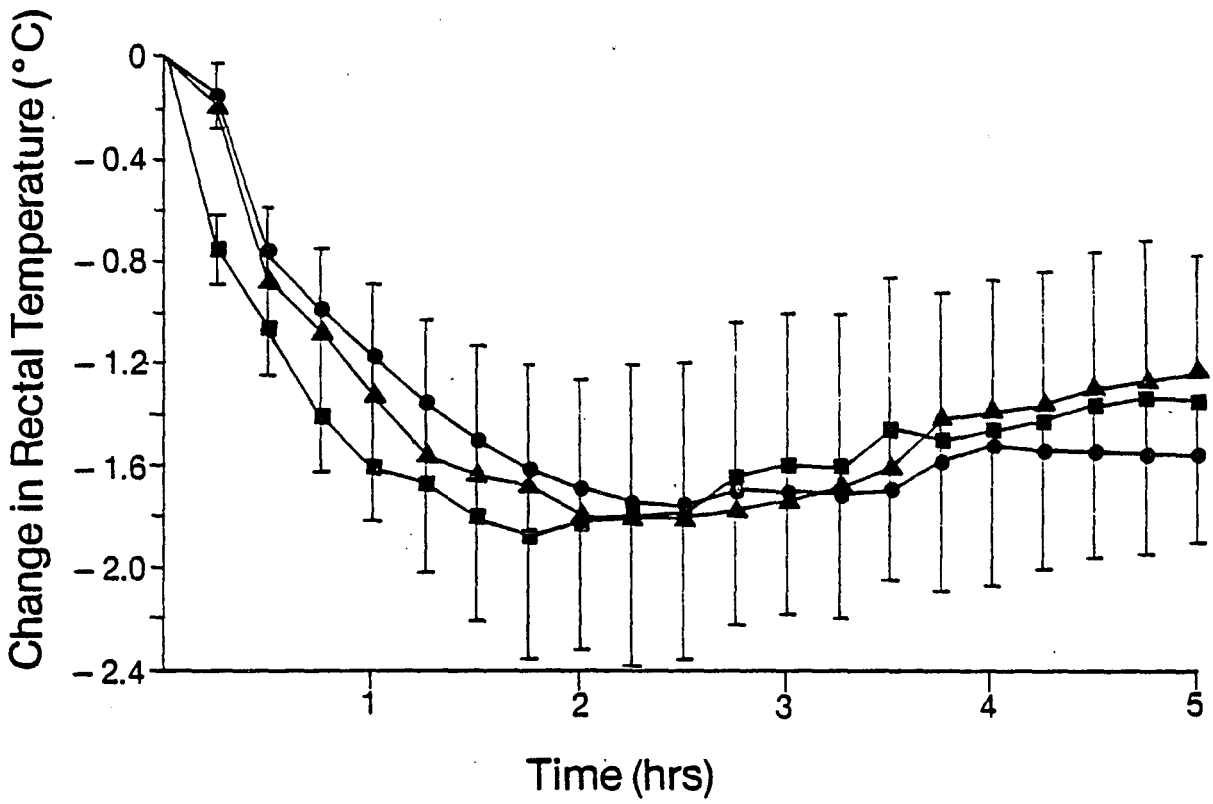

Figure 25. Thermic response to ethanol (1.5 g/kg i.p.). Each point is the mean ± SE of changes from baseline rectal temperatures in 6 or 7 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 17. Effects of ethanol (1.5 g/kg i.p.) on rectal temperature during 5 hours after injection.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	7	275 ± 12	37.0 ± 0.2	- 7.9 ± 2.2	- 2.1 ± 0.4	0.1 ± 0.1	0.2 ± 0.1
Mature	6	340 ± 3	37.0 ± 0.1	- 7.0 ± 0.6	- 2.0 ± 0.2	0.1 ± 0.1	0.0 ± 0.0
Senescent	7	334 ± 12	36.3 ± 0.2	- 7.6 ± 2.4	- 2.0 ± 0.5	0.1 ± 0.1	0.1 ± 0.1
$F_{2,17}$=		10.7	4.2	0.1	0.1	0.7	3.2
Significance ($P \leq .05$)		*	*				

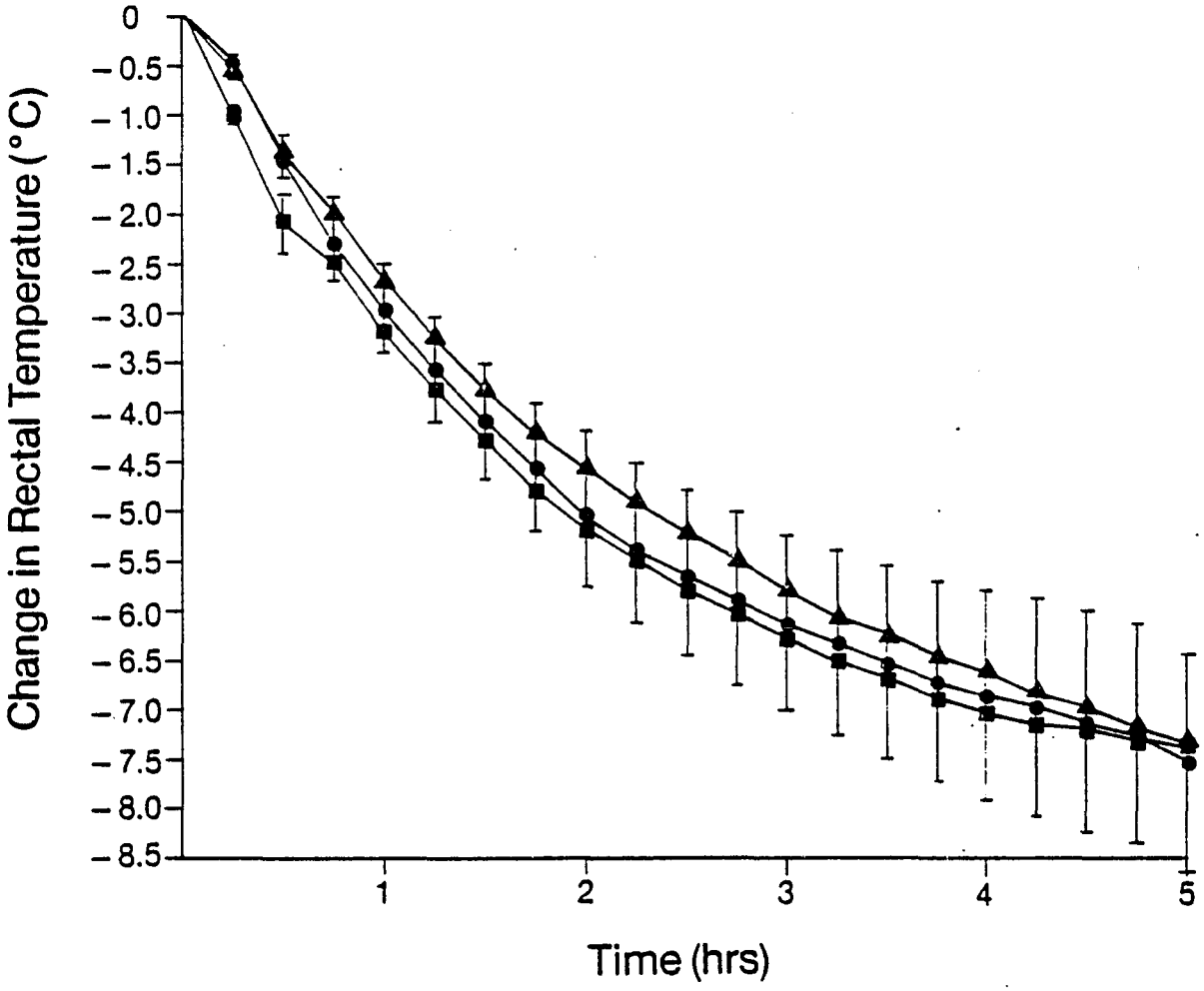

Figure 26. Thermic response to ethanol (3 g/kg i.p.). Each point is the mean ± SE of changes from baseline rectal temperatures in 4 or 5 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 18. Effects of ethanol (3 g/kg i.p.) on rectal temperature during 5 hours after injection.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	5	227 ± 7	36.0 ± 0.6	-25.7 ± 3.8	- 7.5 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
Mature	5	343 ± 26	36.7 ± 0.2	-24.4 ± 7.3	- 7.3 ± 0.8	0.0 ± 0.0	0.0 ± 0.0
Senescent	4	286 ± 18	35.2 ± 0.5	-27.0 ± 2.0	- 7.4 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
$F_{2,11}$=		11.4	2.5	0.2	0.1	-	-
Significance (P≤.05)		*					

Norepinephrine

Norepinephrine (75 ug) injected into the lateral ventricle at an ambient temperature of 22.4 to 23.0 °C caused hypothermia in all groups (Figure 27). The distilled water vehicle had no effect on temperatures. The nadir occurred between 30 minutes and one hour and return to basal temperatures was nearly complete at 3 hours. Table 19 summarizes the thermic data. This dose of norepinephrine elicited vocal responses in all age groups approximately 15 minutes after injection.

Acetylcholine

In rats naive to treatment, acetylcholine (250 ug) caused a transient decrease in rectal temperature of 1 to 2 °C in all age groups at an ambient temperature of 21.8 to 22.2 °C (Figure 28). Time of maximum hypothermia was approximately 30 minutes in all age groups. Young rats were less responsive to the early hypothermic effects of acetylcholine than the mature and senescent rats (Table 20).

Dopamine

Injection of dopamine (100 ug i.c.v.) at an ambient temperature of 21.8 to 22.3 °C resulted in a transient hypothermia in all groups (Figure 29). The magnitude of the decrease in rectal temperature was 1 to 2 °C and the minimum temperatures occurred 30 minutes after injection. Recovery was essentially complete by 1.5 hours after injection. There were no age-related differences in

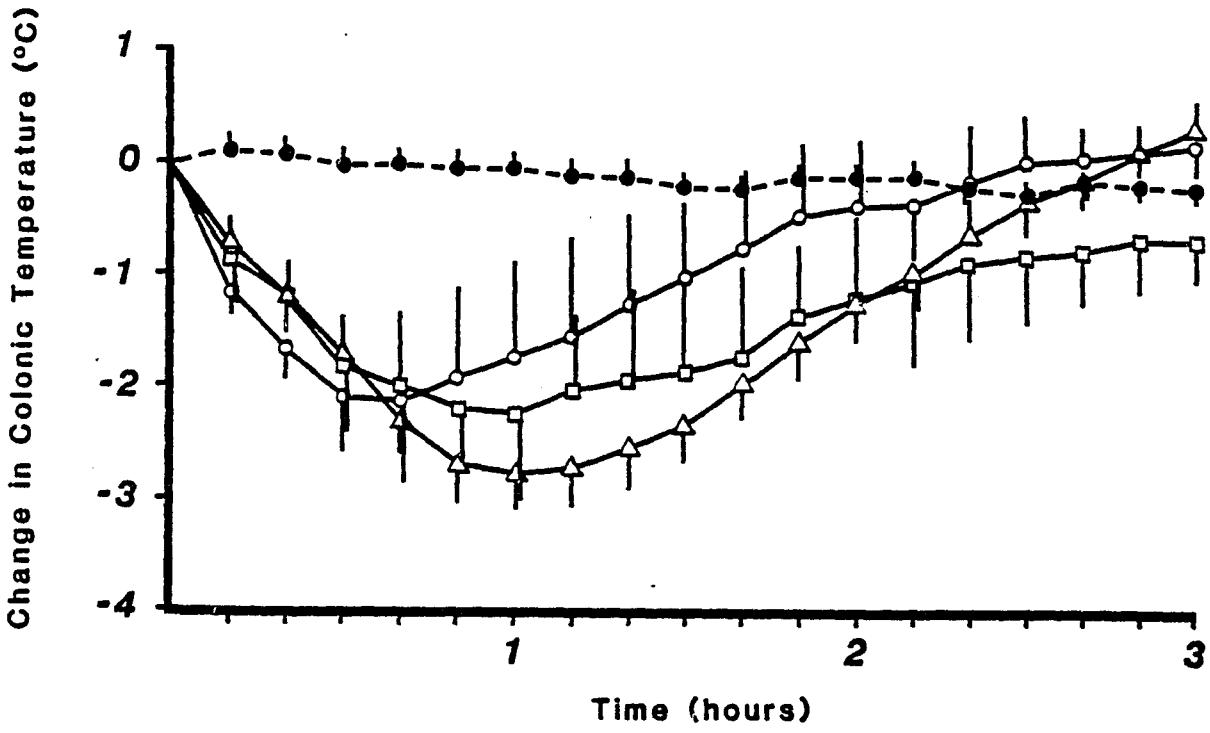

Figure 27. Thermic response to norepinephrine (75 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 4 or 5 rats. Open circles, triangles and squares represent young, mature and senescent groups, respectively. Closed circles represent a group of 2 each age given distilled water.

Table 19. Effects of norepinephrine (75 ug i.c.v.) on rectal temperature during 2 hours after injection.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	4	292 ± 8	37.0 ± 0.2	- 2.7 ± 1.0	- 2.4 ± 0.6	0.2 ± 0.1	0.2 ± 0.1
Mature	5	386 ± 12	36.8 ± 0.1	- 4.0 ± 0.6	- 2.9 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Senescent	4	367 ± 9	35.6 ± 0.6	- 3.5 ± 1.5	- 2.5 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
$F_{2,10}$=		24.0	4.9	0.7	0.2	6.0	2.2
Significance ($P \leq .05$)		*	*			*	

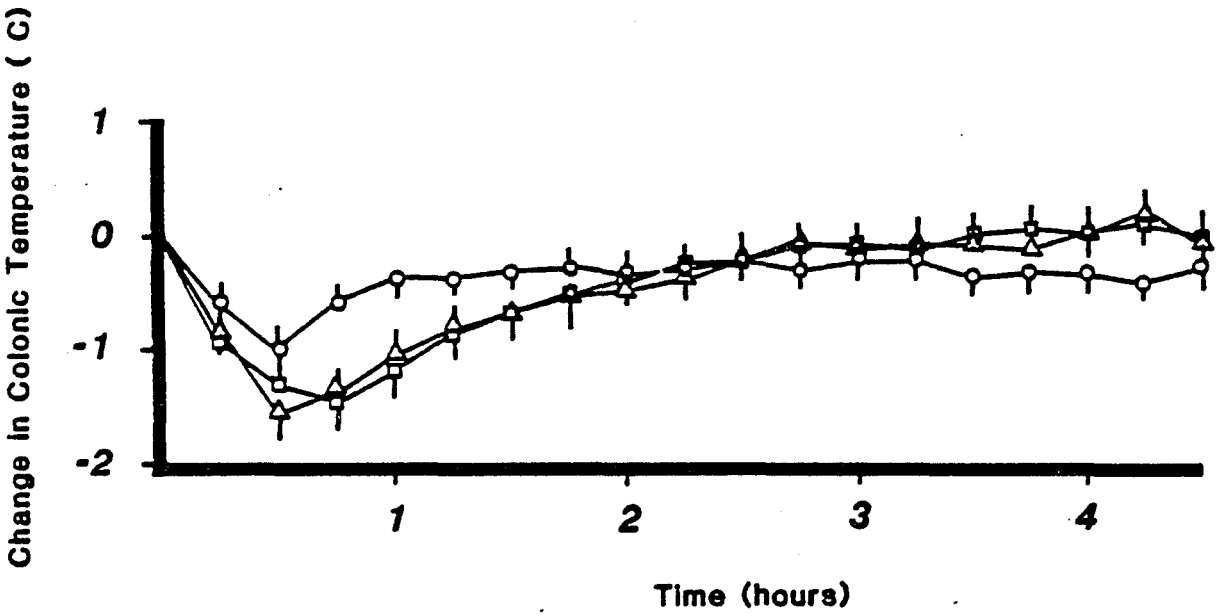

Figure 28. Thermic response to acetylcholine (250 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 8 or 9 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

Table 20. Effects of acetylcholine (250 ug i.c.v.) on rectal temperature during 2 hours after injection.

Group	N	Weight (g±SE)	Baseline (^{o}C±SE)	Neg TRI ($^{o}C \cdot hr$±SE)	Max Hypo (^{o}C±SE)	Pos TRI ($^{o}C \cdot hr$±SE)	Max Hyper (^{o}C±SE)
Young	9	275 ± 4	37.5 ± 0.1	- 1.0 ± 0.2	- 1.0 ± 0.1	0.1 ± 0.1	0.8 ± 0.7
Mature	9	331 ± 7	36.9 ± 0.2	- 1.7 ± 0.2	- 1.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Senescent	8	352 ± 5	36.3 ± 0.2	- 1.9 ± 0.4	- 1.7 ± 0.2	0.0 ± 0.0	0.1 ± 0.1
$F_{2,23}$=		53.6	15.9	4.2	9.2	2.4	1.2
Significance (P≤.05)		*	*	*	*		

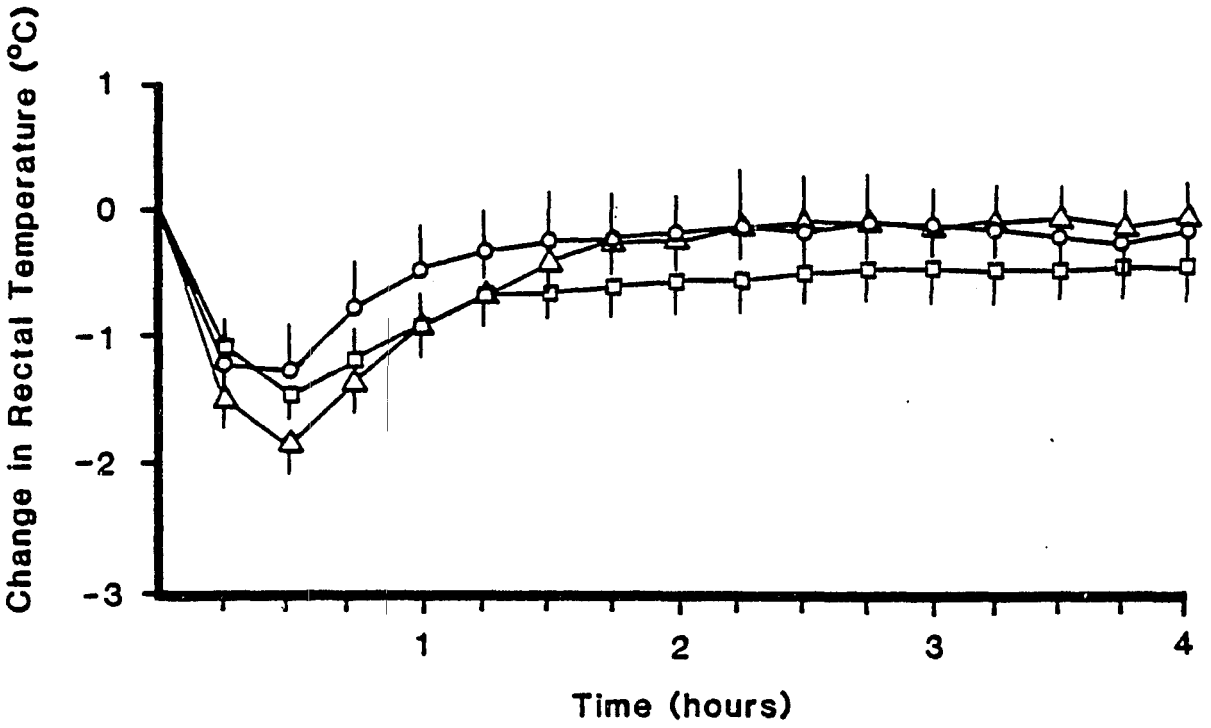

Figure 29. Thermic response to dopamine (100 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 7 or 8 rats. Circles, triangles and squares represent young, mature and senescent groups respectively.

response (Table 21). Table 22 summarizes the effects of ethanol and neurotransmitters.

Single Dose Tolerance

Single dose tolerance to morphine and transmitters was investigated for age-related differences. Tolerance to the thermic effects of a drug was determined to be acquired when the second injection of a drug caused a diminished response compared to the first injection.

Morphine

Tolerance due to one dose of morphine was investigated after subcutaneous and intracerebroventricular administration. A low dose of morphine (5 mg/kg s.c.) resulted in the acquisition of tolerance only in the young and mature groups (Figure 30). There were no differences in response when saline was repeated under the same conditions. Tolerance to the brief hypothermia during the first 1.5 hours after injection was present only in the young and mature groups (Table 23). After the first 1.5 hours, none of the groups responded differently to the second dose (Table 24).

With a larger dose of morphine (25 mg/kg s.c.), all groups responded to both injections with hypothermia which was most pronounced between 2 and 3 hours post-injection (Figure 31). With the second injection, all groups recovered from the hypothermia more quickly and exhibited rebound hyperthermia by approximately 4 hours after injection. Tolerance in the young group was most marked. The

Table 21. Effects of dopamine (100 ug i.c.v.) on rectal temperature during 2 hours after injection.

Group	N	Weight (g±SE)	Baseline (^{o}C±SE)	Neg TRI (^{o}C·hr±SE)	Max Hypo (^{o}C±SE)	Pos TRI (^{o}C·hr±SE)	Max Hyper (^{o}C±SE)
Young	8	319 ± 5	36.9 ± 0.1	- 1.4 ± 0.5	- 1.5 ± 0.3	0.2 ± 0.1	0.2 ± 0.1
Mature	7	360 ± 7	37.0 ± 0.2	- 1.9 ± 0.3	- 1.8 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
Senescent	7	346 ± 13	36.2 ± 0.1	- 1.8 ± 0.3	- 1.5 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
$F_{2,19}$=		5.6	8.0	0.5	0.6	1.9	2.1
Significance (P≤.05)		*	*				

Table 22. Summary of hypothermic effects of ethanol and transmitters.

Parameter	Age-related difference	Response of senescent vs young
Ethanol	No	-
Norepinephrine	No	-
Acetylcholine	Yes	Increased
Dopamine	No	-

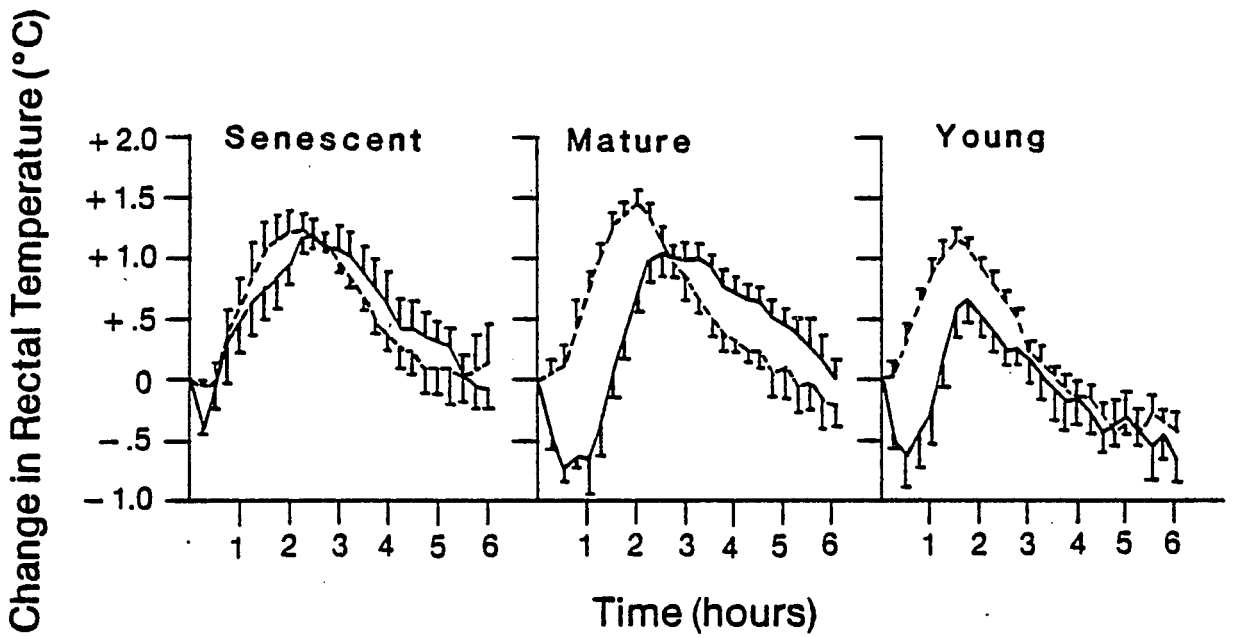

Figure 30. Single dose tolerance to morphine (5 mg/kg s.c.). Each point is the mean ± SE of changes from baseline rectal temperatures in 4 or 5 rats. Solid lines and dashed lines represent the thermic response to the first and second doses, respectively.

Table 23. Single dose tolerance to morphine (5 mg/kg s.c.) during the first 1.5 hours after injection.

Group	N	Hypothermic TRI (°C·hr ± SE)			Hyperthermic TRI (°C·hr ± SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	5	-0.6 ± 0.2	-0.0 ± 0.0	2.2*	0.1 ± 0.1	1.0 ± 0.1	7.5*
Mature	5	-0.8 ± 0.2	-0.0 ± 0.0	3.6*	0.1 ± 0.1	0.9 ± 0.2	3.7*
Senescent	4	-0.2 ± 0.1	-0.1 ± 0.0	1.1	0.2 ± 0.1	0.7 ± 0.3	0.3

* Significant at $P \leq .05$

Table 24. Single dose tolerance to morphine (5 mg/kg s.c.) from 1.5 to 6 hours after injection. Same experiments as Table 20.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	5	-1.0 ± 0.5	-0.7 ± 0.2	0.6	0.8 ± 0.3	1.4 ± 0.2	1.3
Mature	5	-0.1 ± 0.1	-0.3 ± 0.2	1.0	3.0 ± 0.3	2.7 ± 0.5	0.5
Senescent	4	-0.1 ± 0.1	-0.3 ± 0.2	1.0	2.9 ± 0.4	3.0 ± 0.5	0.2

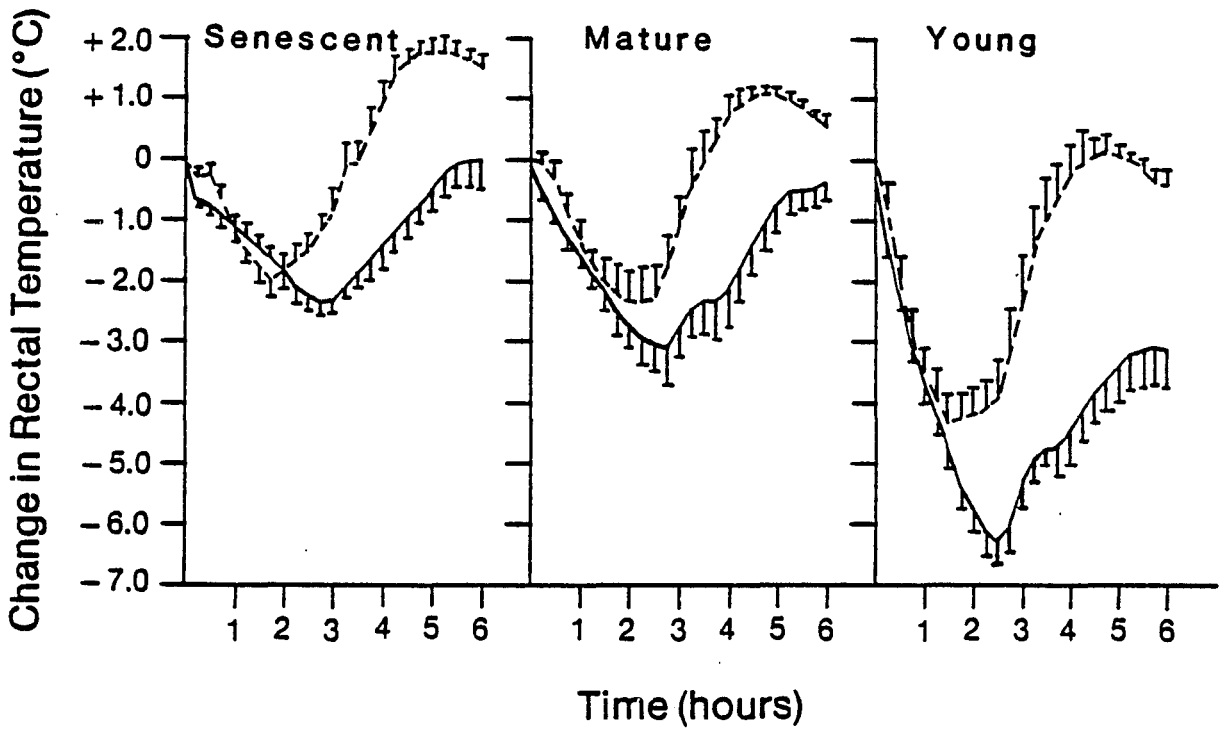

Figure 31. Single dose tolerance to morphine (25 mg/kg s.c.). Each point is the mean ± SE of changes from baseline rectal temperatures in 6 or 7 rats. Solid lines and dashed lines represent the thermic response to the first and second doses, respectively.

maximum decrease in rectal temperature from baseline was less with the second dose only in the young group. The difference in hypothermic TRIs between the first and second doses in the young group (13.7 $^{o}C \cdot hr$) was 2 to 3 times the change due to tolerance in the mature and senescent groups (6.4 and 4.0 $^{o}C \cdot hr$, respectively). All groups had increased hyperthermic response with the second dose (Table 25).

With intracerebroventricularly administered morphine (100 ug), single dose tolerance was exhibited only in the young and mature groups (Figure 32). The hypothermic responses to this dose were almost abolished in the young and mature groups; however, the senescent rats had a hypothermia of approximately 1 ^{o}C with both doses. In the young and mature groups, the hypothermic TRIs were decreased by 3.2 and 3.6 $^{o}C \cdot hr$, respectively (Table 26).

A larger dose of morphine (150 ug i.c.v.) caused tolerance in all groups (Figure 33). The second dose resulted in hyperthermia in the young and mature groups. In the senescent group, hypothermia was still the predominant response, however the magnitude was decreased (Table 27).

Norepinephrine

The first dose of norepinephrine (75 ug i.c.v.) did not cause tolerance in any age group. Similar to the initial response, the second dose caused a hypothermia of 1.5 to 3 ^{o}C with the nadir between 1 and 2 hours (Figure 34). Neither the hyperthermic nor the

Table 25. Single dose tolerance to morphine (25 mg/kg s.c.) during 6 hours after injection.

Group	N	Hypothermic TRI (°C·hr ± SE)			Hyperthermic TRI (°C·hr ± SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	7	-25.1 ± 1.8	-11.9 ± 1.9	5.0*	0.0 ± 0.0	0.4 ± 0.1	2.8*
Mature	6	-10.8 ± 1.8	-4.4 ± 1.4	2.8*	0.3 ± 0.3	2.8 ± 0.5	8.5*
Senescent	6	-7.9 ± 1.3	-3.9 ± 0.5	3.1*	0.4 ± 0.1	3.8 ± 0.4	7.4*

* Significant at $P \leq .05$

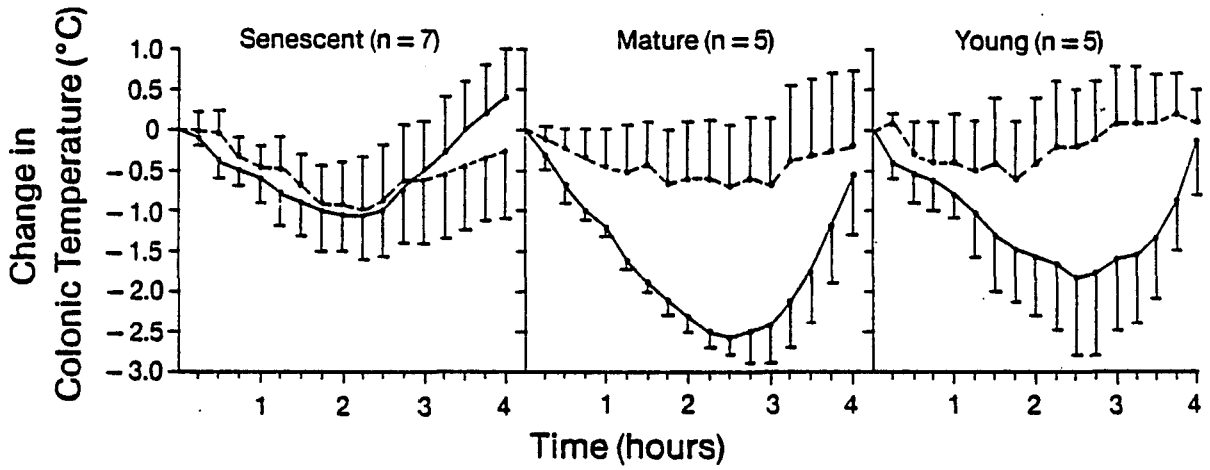

Figure 32. Single dose tolerance to morphine (100 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 5 to 7 rats. Solid lines and dashed lines represent the thermic response to the first and second doses, respectively.

Table 26. Single dose tolerance to morphine (100 ug i.c.v.) during 4 hours after injection.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	5	-5.7 ± 1.6	-2.5 ± 1.3	1.5	1.2 ± 0.9	1.9 ± 0.7	-0.6
Mature	5	-6.9 ± 1.1	-3.3 ± 2.0	1.6	0.4 ± 0.3	1.5 ± 1.1	-1.0
Senescent	7	-3.1 ± 1.4	-4.0 ± 1.4	-0.4	1.3 ± 0.6	1.8 ± 0.9	-0.5

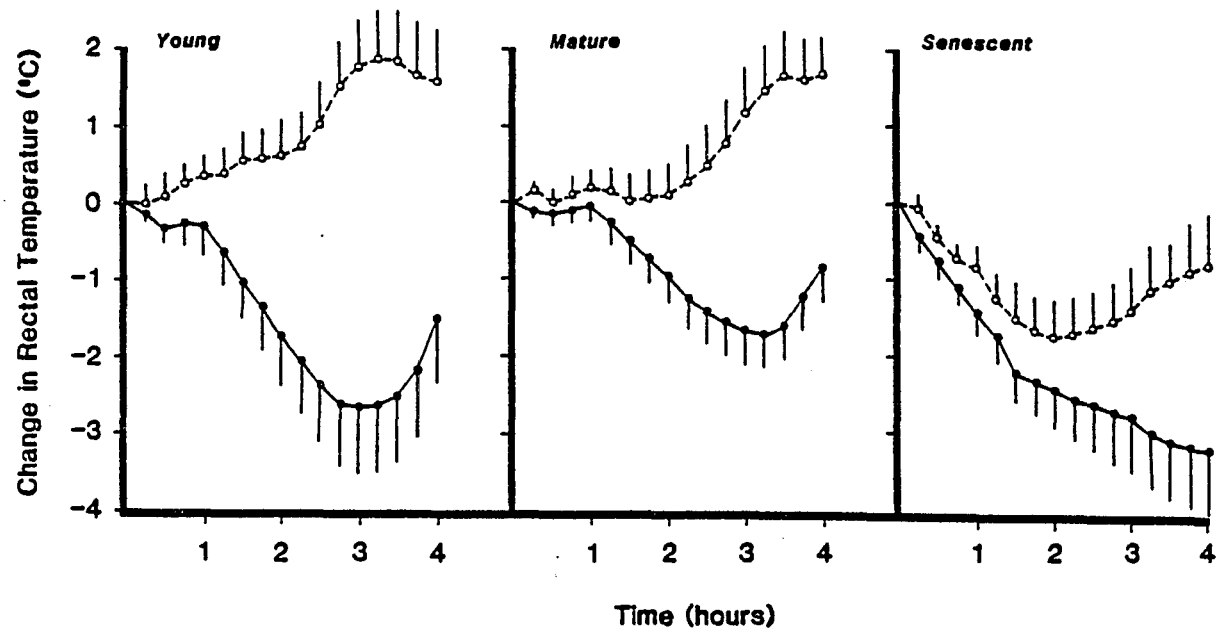

Figure 33. Single dose tolerance to morphine (150 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 6 to 8 rats. Solid lines and dashed lines represent the thermic response to the first and second doses, respectively.

Table 27. Single dose tolerance to morphine (150 ug i.c.v.) during 4 hours after injection.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	6	-7.5 ± 3.0	-0.8 ± 0.5	2.2*	2.6 ± 1.0	5.7 ± 1.8	1.5
Mature	8	-4.9 ± 1.3	-1.1 ± 0.5	2.7*	0.7 ± 0.4	4.9 ± 1.4	-2.8*
Senescent	7	-10.8 ± 2.2	-5.2 ± 1.8	2.0*	0.6 ± 0.5	1.2 ± 0.6	-0.8

* Significant at $P \leq .05$

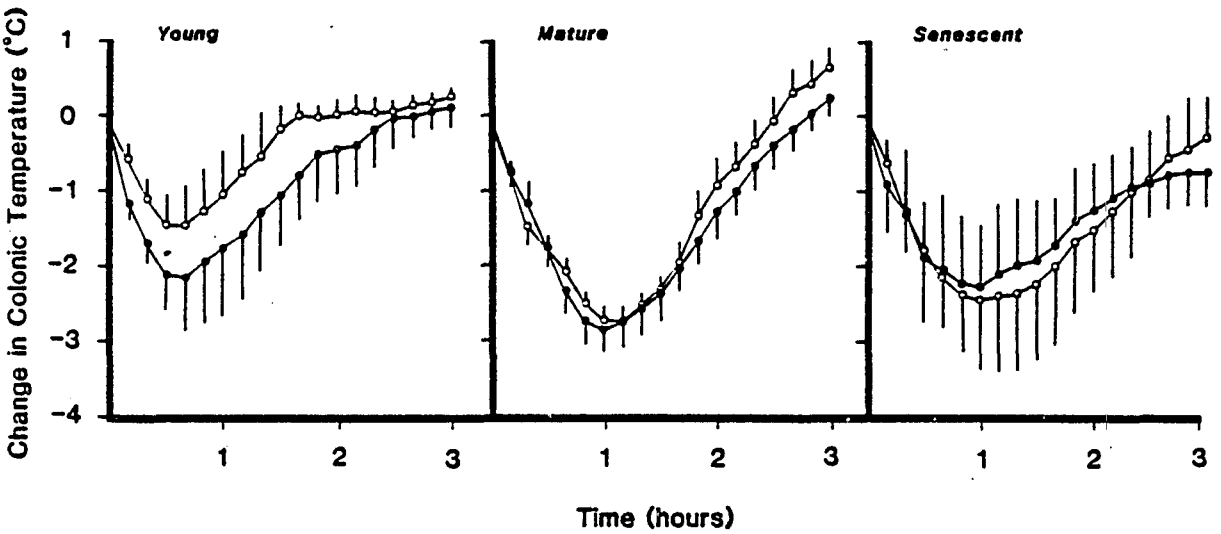

Figure 34. Single dose tolerance to norepinephrine (75 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 5 rats. Closed circles and open circles represent the thermic response to the first and second doses, respectively.

hypothermic TRIs for the second dose were different from the TRIs due to the first dose (Table 28).

Acetylcholine

Acetylcholine (250 ug i.c.v.) caused less of a hypothermic response and more rapid recovery with a hyperthermic component with the second dose than with the first (Figure 35). During the first 2 hours the hypothermic TRIs were less in the young and mature groups with the second dose (Table 29).

Dopamine

The first dose of dopamine (100 ug i.c.v.) did not cause tolerance in any age group (Figure 36). First and second hypothermic and hyperthermic TRIs were almost identical in all age groups (Table 30). Table 31 summarizes single dose tolerance data.

Chronic Morphine Tolerance

The effect of tolerance induction by implantation of morphine pellets was investigated by naloxone-precipitated withdrawal or by subsequent administration of subcutaneous or intracerebroventricular morphine. When baselines of morphine-pelleted rats were compared with baselines of non-pelleted rats utilized in separate experiments, morphine pellet implanted rats had baselines that were 0.5 to 1.0 ^{o}C higher than the baselines of rats which were not pelleted (Table 32). The same effect of morphine pellet implantation on baselines was seen in experiments in which both placebo and morphine pellets were implanted. Basal temperatures of mature rats implanted with either 3

Table 28. Single dose tolerance to norepinephrine (75 ug i.c.v.) during 2 hours after injection.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	5	-2.7 ± 1.0	-1.3 ± 0.6	1.1	0.3 ± 0.2	0.2 ± 0.1	0.6
Mature	5	-4.0 ± 0.6	-3.9 ± 0.3	0.3	0.0 ± 0.0	0.0 ± 0.0	0.2
Senescent	5	-3.5 ± 1.5	-4.0 ± 1.4	-0.2	0.0 ± 0.0	0.2 ± 0.2	-0.9

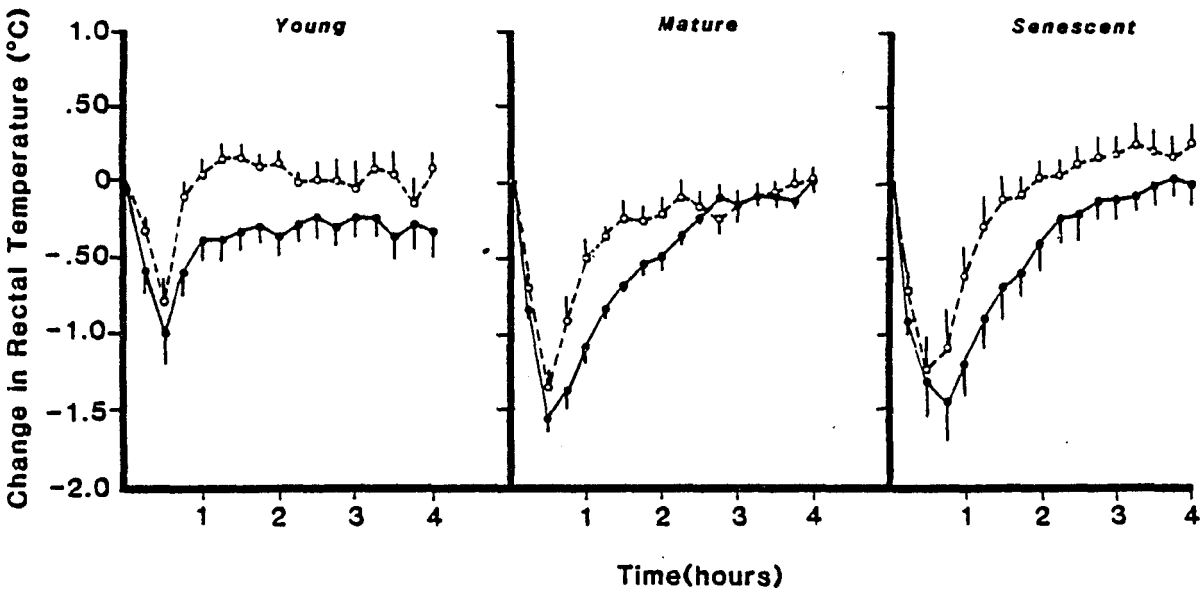

Figure 35. Single dose tolerance to acetylcholine (250 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 7 to 9 rats. Solid lines and dashed lines represent the thermic response to the first and second doses, respectively.

Table 29. Single dose tolerance to acetylcholine (250 ug i.c.v.) during 2 hours after injection.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	9	-1.0 ± 0.2	-0.4 ± 0.2	2.4*	0.1 ± 0.0	0.2 ± 0.1	2.7*
Mature	8	-1.8 ± 0.1	-1.3 ± 0.2	2.8*	0.0 ± 0.0	0.0 ± 0.0	1.4
Senescent	7	-1.7 ± 0.3	-1.2 ± 0.3	1.2	0.0 ± 0.0	0.1 ± 0.1	1.3

* Significant at $P \leq .05$

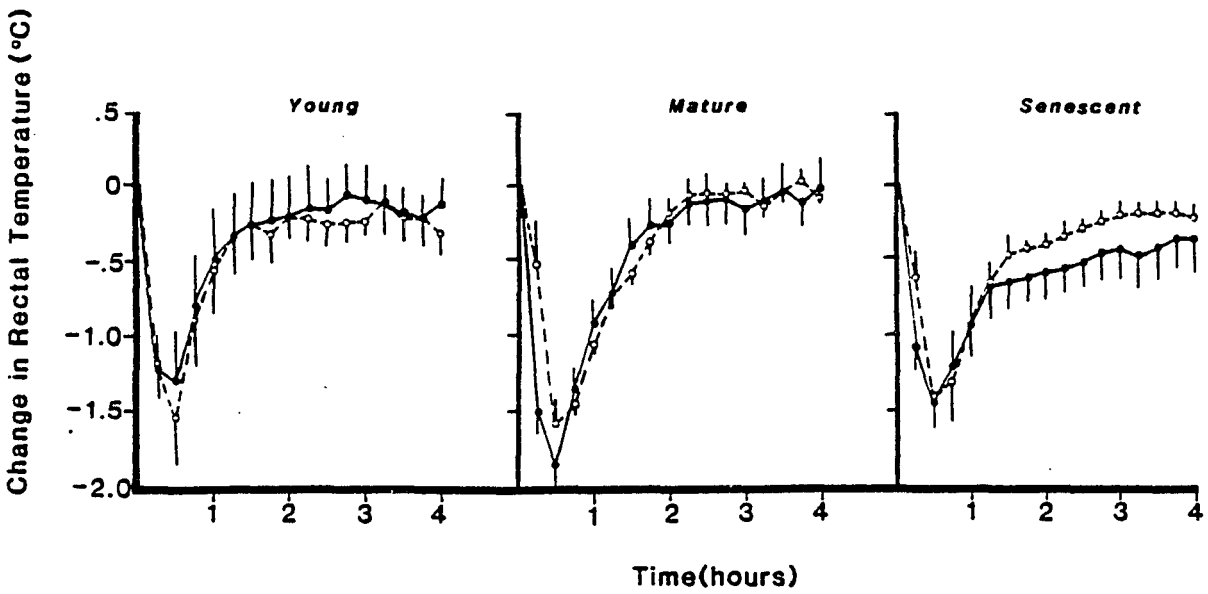

Figure 36. Single dose tolerance to dopamine (100 ug i.c.v.). Each point is the mean ± SE of changes from baseline rectal temperatures in 5 to 7 rats. Solid lines and dashed lines represent the thermic response to the first and second doses, respectively.

Table 30. Single dose tolerance to dopamine (100 ug i.c.v.) during 2 hours after injection.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	7	-1.3 ± 0.6	-1.4 ± 0.4	0.2	0.2 ± 0.1	0.1 ± 0.1	1.1
Mature	5	-1.7 ± 0.3	-1.6 ± 0.1	0.2	0.0 ± 0.0	0.0 ± 0.0	-0.2
Senescent	6	-1.7 ± 0.3	-1.5 ± 0.2	0.7	0.1 ± 0.1	0.1 ± 0.1	-0.3

Table 31. Summary of tolerance induction by a single dose. Norepinephrine and dopamine did not cause tolerance.

Parameter	Age-related difference	Response of senescent vs young
Morphine (s.c.) (low dose)	Yes	Decreased
Morphine (s.c.) (high dose)	No	-
Morphine (i.c.v.) (low dose)	No	-
Acetylcholine (i.c.v.)	Yes	Decreased

Table 32. Effects of morphine pellets on basal temperatures 3 days after implantation. Basal temperatures were compiled from Tables 3,5,29 and 30.

Group	Naive N	Naive Baseline (°C ± SE)	Tolerant N	Tolerant Baseline (°C ±)	t
Young	12	36.1 ± 0.2	10	36.7 ± 0.2	-2.1*
Mature	11	36.1 ± 0.3	10	37.0 ± 0.3	-2.4*
Senescent	12	36.0 ± 0.2	10	37.0 ± 0.1	-4.7*

* $P \leq .05$, t-test

placebo or 3 morphine pellets (Naloxone experiment) were 36.6 ± 0.6 and 37.1 ± 0.7 °C, respectively, 3 days after pellet implantation. Basal temperatures of young rats implanted with either 2 placebo or 2 morphine pellets (subcutaneous morphine test) were 36.1 ± 0.4 and 37.0 ± °C, respectively, three days after implantation.

Due to the smaller weight of the rats in the young group, 2 morphine pellets were implanted in each young rat and 3 pellets were implanted in each mature and senescent rat. The mean implanted dose of morphine was 549, 648 and 643 mg/kg in the young, mature and senescent groups, respectively.

Naloxone-precipitated Withdrawal

Naloxone (1 mg/kg s.c.) caused hypothermia of 4 to 5 °C in morphine-pelleted rats with a nadir at 1.5 to 3 hours and had no effect on the rectal temperatures of placebo-pelleted rats (Figure 37). No age-related differences in naloxone-precipitated hypothermic TRIs were present (Table 33).

Subcutaneous Morphine Test

Morphine (25 mg/kg) caused less hypothermia in morphine pellet implanted rats than in placebo implanted rats (Figure 38). In the mature and senescent rats the predominant response was hyperthermia. Young morphine-pelleted rats responded with approximately one-half the hypothermic TRI of the young placebo-pelleted rats (Table 34). Pellets had little effect on body weight during the 72 hours of implantation. Changes in body weight were -1, -2, -4 and +1 percent

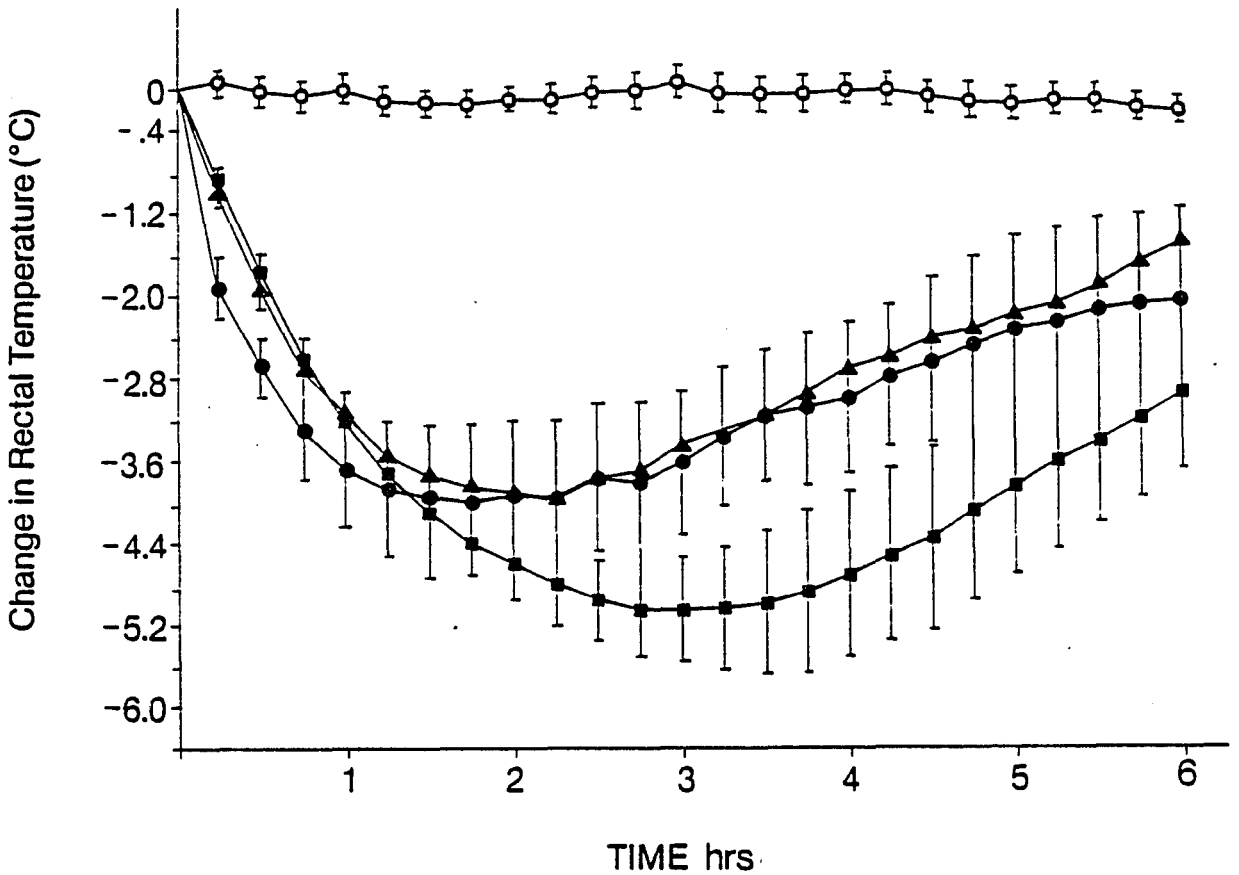

Figure 37. Thermic response to naloxone (1 mg/kg s.c.). in placebo and morphine pellet implanted rats. Each point is the mean ± SE of changes from baseline rectal temperatures in 4 or 5 rats. Solid circles, triangles and squares represent young, mature and senescent rats respectively which were implanted with morphine pellets. Open circles represent mature rats which were implanted with placebo pellets.

Table 33. Precipitated withdrawal with naloxone (1 mg/kg s.c.) during 6 hours after injection. Rats were implanted with placebo or morphine pellets 72 hours prior to injection.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	4	263 ± 6	36.4 ± 0.3	-18.5 ± 4.6	- 4.2 ± 0.8	0.0 ± 0.0	0.0 ± 0.0
Mature	5	347 ± 12	37.1 ± 0.7	-16.8 ± 2.0	- 4.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Senescent	5	353 ± 13	36.8 ± 0.2	-23.7 ± 3.2	- 5.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
Mature (Placebo)	5	381 ± 11	36.6 ± 0.2	- 0.8 ± 0.5	- 0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
$F_{3,15}$=		14.7	1.2	13.4	23.5	5.2	4.7
Significance ($P \leq .05$)		*		*	*	*	*

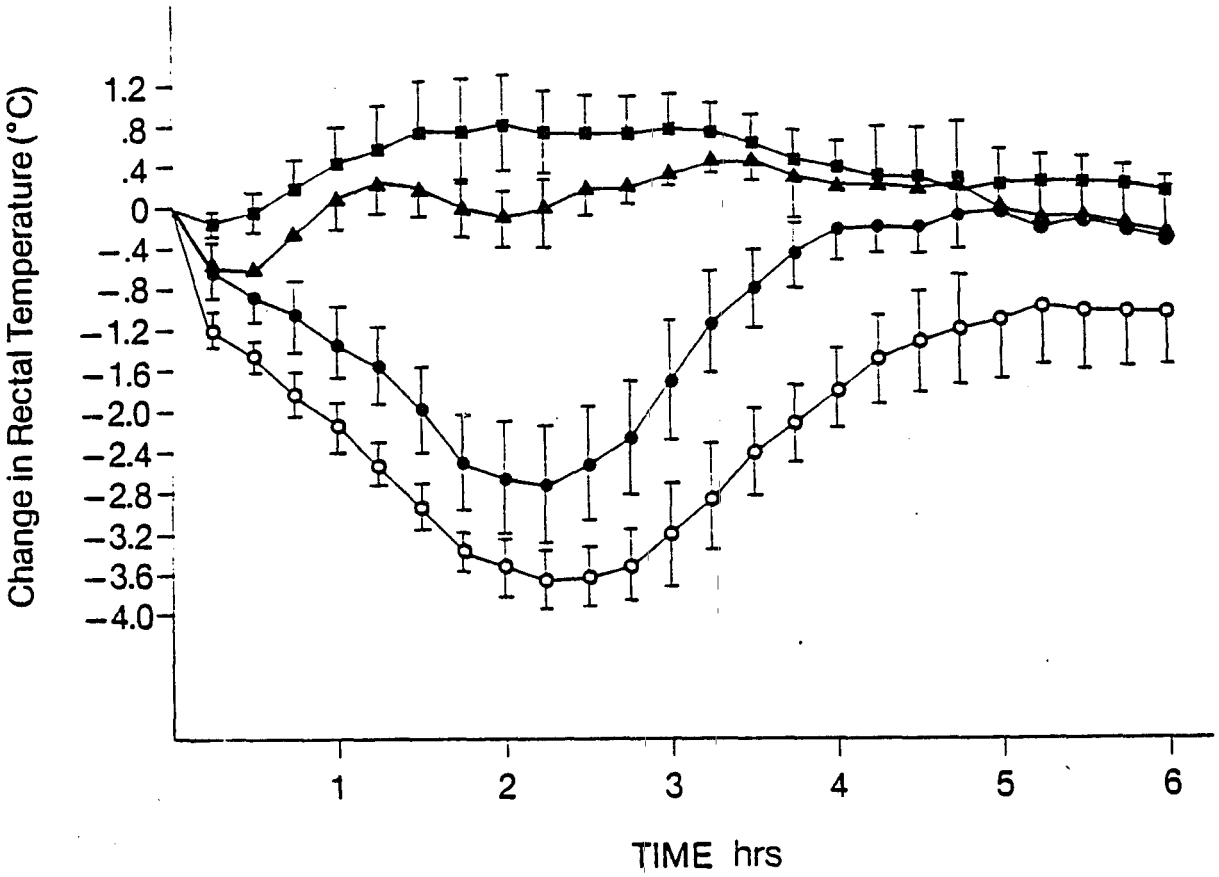

Figure 38. Thermic response to morphine (25 mg/kg s.c.) in placebo and morphine pellet implanted rats. Each point is the mean ± SE of changes from baseline rectal temperatures in 5 rats. Solid circles, triangles and squares represent young, mature and senescent rats, respectively which were implanted with morphine pellets. Open circles represent young rats which were implanted with placebo pellets.

Table 34. Effect of morphine (25 mg/kg s.c.) on rectal temperature of rats implanted with morphine pellets for 72 hours. Rectal temperatures were measured for 6 hours after injection.

Group	N	Weight (g±SE)	Baseline (°C±SE)	Neg TRI (°C·hr±SE)	Max Hypo (°C±SE)	Pos TRI (°C·hr±SE)	Max Hyper (°C±SE)
Young	5	278 ± 8	37.0 ± 0.3	- 6.7 ± 1.6	- 0.8 ± 0.1	3.1 ± 1.2	0.3 ± 0.1
Mature	5	347 ± 9	36.9 ± 0.4	- 1.8 ± 0.8	- 0.8 ± 0.1	2.8 ± 1.0	1.0 ± 0.3
Senescent	5	351 ± 11	37.2 ± 0.1	- 0.6 ± 0.4	- 2.8 ± 0.5	0.5 ± 0.2	1.1 ± 0.3
Young (Placebo)	5	290 ± 11	36.1 ± 0.4	-13.1 ± 1.8	- 3.8 ± 0.3	0.1 ± 0.1	0.1 ± 0.1
$F_{3,16}$=		60.6	3.0	20.2	26.4	4.0	4.3
Significance ($P \leq .05$)		*		*	*	*	*

for young, mature, senescent and placebo-pelleted young rats, respectively. During the 9 hour period of restraint the rats lost 2 to 4 percent of their body weight. When the hypothermic responses in this experiment were compared with the hypothermic responses in naive rats which received the same dose of morphine (Table 6), the decreases in hypothermic TRIs due to tolerance were 12.1, 9.8 and 7.3 $^{o}C \cdot hr$ for young, mature and senescent groups, respectively.

Intracerebroventricular Morphine Test

Chronic tolerance was tested with i.c.v. administration of morphine (150 ug) using the protocol shown in Figure 5. When 150 ug of morphine was injected in morphine-pelleted rats, the hypothermic response was attenuated and an overshoot hyperthermia was apparent on recovery (Figure 39). Vocalization was not or was only slightly diminished by pellet implantation. Table 35 shows that the young and senescent groups had attenuated hypothermic TRIs with the second dose.

Morphine Cross-tolerance

The effect of morphine tolerance, induced by implantation of morphine pellets subcutaneously for 72 hours, on the thermic effects of norepinephrine, acetylcholine and dopamine was investigated using the protocol previously described (Figure 5).

Norepinephrine

Tolerance to morphine induced tolerance to norepinephrine only in mature and senescent groups (Figure 40). The thermic response of

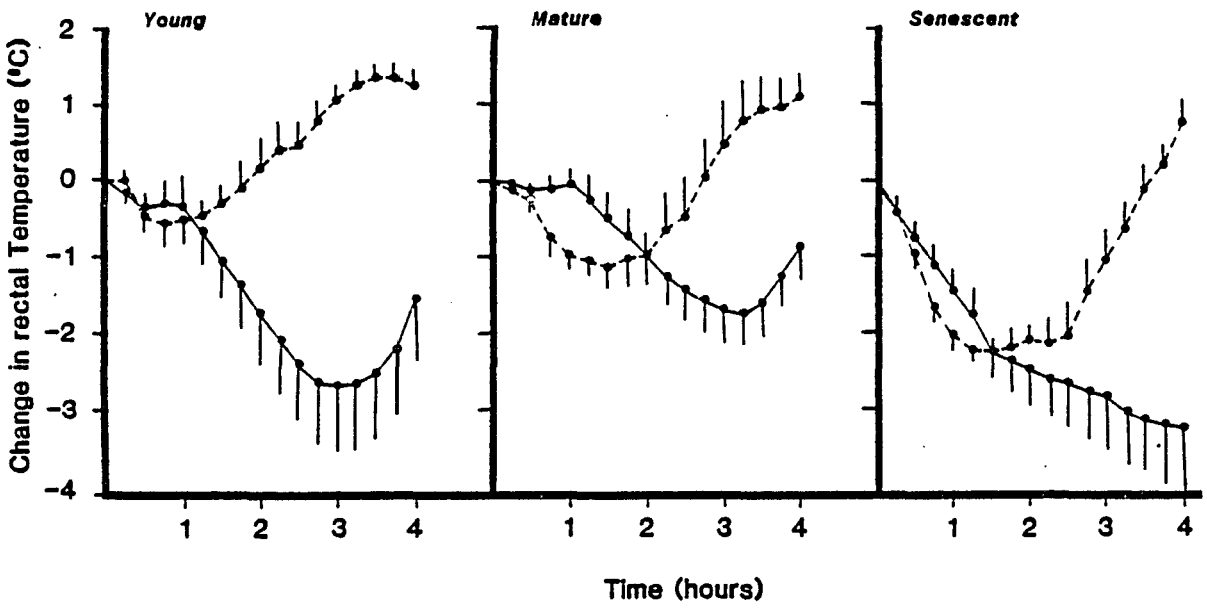

Figure 39. Thermic response to morphine (150 ug i.c.v.) in morphine pellet implanted rats. Each point is the mean ± SE of changes from baseline rectal temperatures in 4 to 7 rats. Solid lines and dashed lines represent the thermic response in the naive and morphine tolerant states respectively.

Table 35. Effect of morphine (150 ug i.c.v.) on rectal temperatures of rats implanted with morphine pellets for 72 hours. Rectal temperatures were measured for 4 hours after injection.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	5	-7.6 ± 2.3	-0.9 ± 0.3	2.2*	1.4 ± 1.0	2.3 ± 0.4	-0.8
Mature	7	-4.2 ± 1.4	-2.4 ± 0.8	1.1	0.8 ± 0.5	1.6 ± 0.4	-1.3
Senescent	4	-12.5 ± 2.1	-5.4 ± 0.9	3.1*	0.0 ± 0.0	0.4 ± 0.2	-1.7

* Significant at $P \leq .05$

Table 35. Effect of morphine (150 ug i.c.v.) on rectal temperatures of rats implanted with morphine pellets for 72 hours. Rectal temperatures were measured for 4 hours after injection.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	5	-7.6 ± 2.3	-0.9 ± 0.3	2.2*	1.4 ± 1.0	2.3 ± 0.4	-0.8
Mature	7	-4.2 ± 1.4	-2.4 ± 0.8	1.1	0.8 ± 0.5	1.6 ± 0.4	-1.3
Senescent	4	-12.5 ± 2.1	-5.4 ± 0.9	3.1*	0.0 ± 0.0	0.4 ± 0.2	-1.7

* Significant at $P \leq .05$

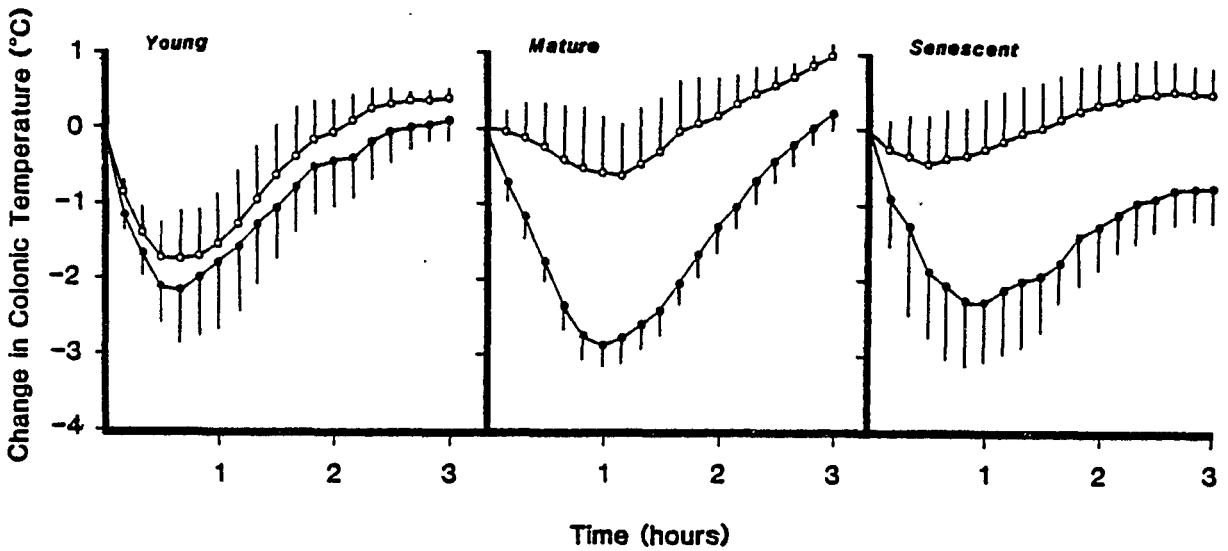

Figure 40. Thermic response to norepinephrine (75 ug i.c.v.) in morphine pellet-implanted rats. Each point is the mean ± SE of changes from baseline rectal temperatures in 4 or 5 rats. Solid circles and open circles represent the thermic response in the naive and morphine tolerant states respectively.

the young rats after morphine tolerance was not different from the response of the young rats when naive. Both the hypothermic and hyperthermic TRIs of the mature and senescent rats were affected by morphine tolerance (Table 36). In contrast, the young rats showed no changes after morphine tolerance.

Acetylcholine

Morphine tolerance affected the acetylcholine responses in all age groups. In all groups the overshoot hyperthermia was greater in magnitude in the tolerant state (Figure 41). During the first 2 hours the hypothermia induced by acetylcholine in the young group was reduced by one-half, but the tolerance did not change the hypothermic TRIs in the mature and senescent groups (Table 37). All groups had a greater hyperthermic response in the tolerant state during the first 2 hours when compared to the non-tolerant state.

Dopamine

Tolerance to morphine caused changes in dopamine-induced thermic responses in the mature and senescent age groups (Figure 42). during the first 2 hours post-injection, the hypothermic TRIs were decreased and hyperthermic TRIs were increased in the mature and senescent groups (Table 38). Table 39 summarizes cross-tolerance data.

Table 36. Effect of norepinephrine (75 ug i.c.v.) on rectal tmeperature of rats implanted with morphine pellets for 72 hours. Data represent the first 2 hours after injection.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	5	-2.7 ± 1.0	-2.2 ± 0.9	0.4	0.3 ± 0.2	0.3 ± 0.1	0.3
Mature	5	-4.0 ± 0.6	-1.4 ± 0.9	2.5*	0.0 ± 0.0	1.0 ± 0.3	3.0*
Senescent	4	-4.3 ± 1.5	-1.1 ± 0.9	1.8*	0.0 ± 0.0	0.8 ± 0.3	2.9*

* Significant at $P \leq .05$

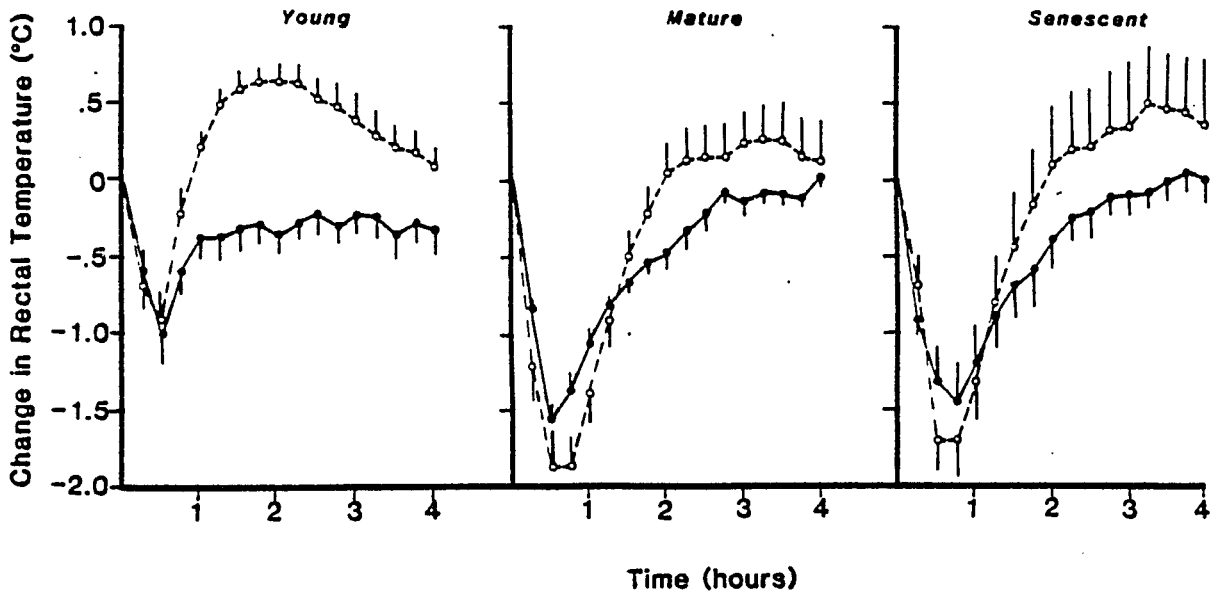

Figure 41. Thermic response to acetylcholine (250 ug i.c.v.) in morphine pellet-implanted rats. Each point is the mean ± SE of changes from baseline rectal temperatures in 7 to 9 rats. Solid lines and dashed lines represent the thermic response in the naive and morphine tolerant states respectively.

Table 37. Effect of acetylcholine (250 ug i.c.v.) on rectal temperature of rats implanted with morphine pellets for 72 hours. Data represent the first 2 hours after injection.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	9	-1.0 ± 0.2	-0.5 ± 0.1	2.5*	0.1 ± 0.0	0.7 ± 0.1	-5.4*
Mature	8	-1.8 ± 0.1	-2.0 ± 0.4	-0.6	0.0 ± 0.0	0.4 ± 0.2	-2.4*
Senescent	7	-1.7 ± 0.3	-1.9 ± 0.4	-0.5	0.0 ± 0.0	0.2 ± 1.0	-2.0*

* Significant at $P \leq .05$

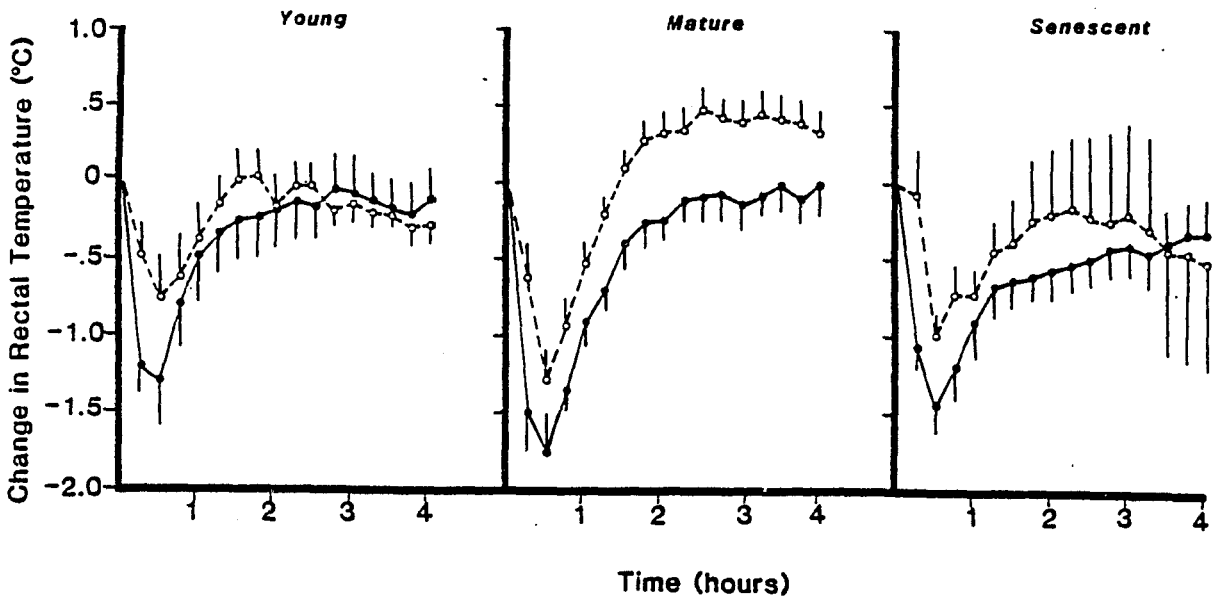

Figure 42. Thermic response to dopamine (100 ug i.c.v.) in morphine pellet-implanted rats. Each point is the mean ± SE of changes from baseline rectal temperatures in 4 to 8 rats. Solid lines and dashed lines represent the thermic response in the naive and morphine tolerant states respectively.

Table 38. Effect of dopamine (100 ug i.c.v.) on rectal temperature of rats implanted with morphine pellets for 72 hours. Data represent the first 2 hours after injection.

Group	N	Hypothermic TRI ($^{o}C \cdot hr \pm$ SE)			Hyperthermic TRI ($^{o}C \cdot hr \pm$ SE)		
		Naive	Non-naive	t	Naive	Non-naive	t
Young	8	-1.4 ± 0.5	-0.8 ± 0.2	0.8	0.2 ± 0.1	0.2 ± 0.1	0.3
Mature	5	-1.7 ± 0.3	-0.9 ± 0.2	2.4*	0.0 ± 0.0	0.2 ± 0.1	-1.9*
Senescent	4	-2.0 ± 0.3	-0.8 ± 0.1	3.6*	0.0 ± 0.0	0.2 ± 0.1	-2.1*

Table 39. Summary of cross-tolerance to morphine. Morphine pellets induced tolerance to morphine in all groups. Naloxone-induced withdrawal showed to age-related differences.

Parameter	Age-related difference	Response of senescent vs young
Norepinephrine	Yes	Increased
Acetylcholine	Yes	Decreased
Dopamine	Yes	Increased

Morphine Analgesia

The morphine-induced increase in latency to respond to heat did not show age-related differences. An analgesic dose of morphine administered intravenously increased the latency by 4 to 5 seconds (Table 40). No differences in baseline latency were present. A larger dose of morphine administered subcutaneously (15 mg/kg) caused 45 per cent more analgesia in the young rats than in the senescent rats, although there were not enough rats for a statistical difference (Table 41). There were no age-related differences in the quality of the response to the heat source and rats in all groups frequently moved their tails more slowly under the influence of morphine.

Morphine Respiratory Depression

The respiratory depressant effect of intravenous morphine was studied in young and senescent rats. Baseline respiratory rates in the senescent rats were approximately 75 percent lower than in the young rats (Table 42 & 43). A low dose of morphine (2.5 mg/kg) depressed respiratory rate maximally at 15 to 30 minutes after injection. This dose depressed respiratory rate approximately 40 percent in each age group (Table 42). A moderate dose of morphine (5 mg/kg i.v.) caused 55 to 65 percent respiratory depression in both age groups (Table 43). A larger dose of morphine (10 mg/kg i.v.) caused cessation of respiration in both rats of each age into which the dose was injected. Morphine changed the normal regular chest

Table 40. Analgesic response to morphine (7.5 mg/kg i.v.) 15 minutes after injection.

Group	N	Baseline Latency (Sec ± SE)	Change (Sec ± SE)
Young	4	6.0 ± 0.2	+ 4.9 ± 0.9
Mature	5	6.1 ± 0.2	+ 4.2 ± 0.8
Senescent	3	5.5 ± 0.6	+ 4.3 ± 1.1
$F_{2,9}$=		0.8	0.2

Table 41. Analgesic response to morphine (15 mg/kg s.c.) 15 minutes after injection.

Group	N	Baseline Latency (Sec ± SE)	Change (Sec ± SE)
Young	5	6.4 ± 0.3	+ 3.8 ± 0.8
Mature	5	6.2 ± 0.3	+ 3.4 ± 0.7
Senescent	5	5.7 ± 0.3	+ 2.1 ± 0.2
$F_{2,9}$=		1.1	1.9

Table 42. Respiratory depression from morphine (2.5 mg/kg i.v.). Respirtory rate was measured for 30 minutes prior to and 2 to 4 hours after injection.

Group	N	Respiratory rate (Breaths/min± SE)	Maximum Change (Percent ± SE)
Young	4	120 ± 10	-43 ±
Senescent	4	87 ± 4	-42 ±
t=		2.9*	0.4

* P≥ .05, t-test

Table 43. Respiratory depression fron morphine (5 mg/kg s.c.). Respiratory rate was measured for 30 minutes prior to and 2 to 4 hours after injection.

Group	N	Respiratory rate (Breaths/min± SE)	Maximum Change (Percent ± SE)
Young	2	107 ± 10	-55 ± 3
Senescent	4	83 ± 3	-63 ± 6
t=		2.8*	0.8

* P≥ .05, t-test

breathing observed during the baseline period into a shallow, irregular abdominal pattern.

Tritiated Water Absorption

The apprearance of tritiated water in the blood and disappearance from the site of injection are shown in Figure 43. No age-related differences in either parameter were apparent. Eighty percent of the radioactivity in the site was gone 9 minutes after injection, and approximately 90 percent of the maximum blood levels were achieved by 9 minutes.

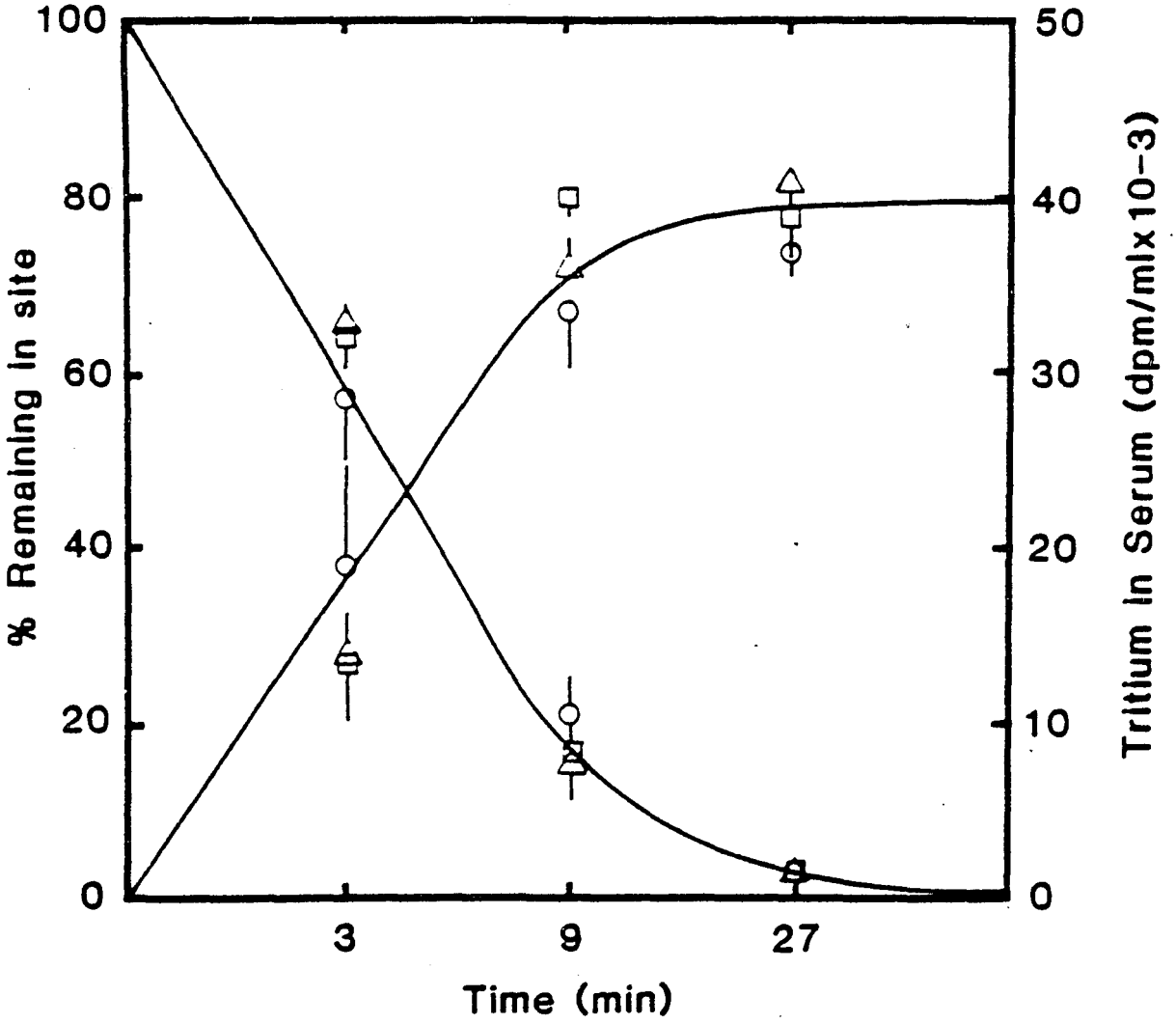

Figure 43. Subcutaneous absorption of tritiated water in Fischer 344 rats. Circles, triangles and squares represent young, mature and senescent groups, respectively.

DISCUSSION

Although some attempts have been made to characterize the age-related differences in certain opiate responses of rodents (Spratto and Dorio, 1978; Saunders et al., 1974 ; Webster, Schuster and Eleftheriou, 1976), the study reported here was the first to characterize the thermoregulatory, analgesic and respiratory depressant effects of acutely administered morphine in rats comparable in age to geriatric humans. The results of this study indicate that the senescent rat has a thermoregulatory system which is less responsive than the thermoregulatory system in young rats. Senescent rats thermoregulate at lower body temperatures than young rats and are less able to compensate for cold and heat stress. Senescent rats were less responsive to the hypothermic effects of subcutaneous morphine, but not i.c.v. morphine. Morphine administered i.c.v. affects only periventricular sites and these studies suggest that age-related differences with subcutaneous morphine are due to differences in sensitivity of a non-periventricular site of action. Systems other than thermoregulation were also affected differently by morphine in the old and young rats, since senescent rats were also less responsive to the analgesic effects of subcutaneous morphine. Age-related differences in opiate response and acquisition of tolerance reflect both changes in adaptability and different compensatory mechanisms with aging.

Several differences in the thermoregulatory systems of aged rats became apparent in these studies. Basal rectal temperatures of the senescent rats in these experiments were a full degree lower than the rectal temperatures of the young rats. Rommelspacher, Schulze and Bold (1974) found a similar 0.8 °C difference in rectal temperatures in female Wistar rats which in this study would be classified as young (3 month) and mature (13 month). This lower "normal" temperature was an indication of decreased metabolic needs of the aged organism, and has also been reported in elderly humans (Exton-Smith, 1964). A decreased body temperature of 1 °C is probably not physiologically significant in rats or humans, ie. does not interfere with normal health. Drugs or thermal stress may however, easily overwhelm a less responsive senescent thermoregulatory system.

In order to determine if cold stress affected rats of the three age groups similarly, the groups were exposed to a rather extreme cold environment (as evidenced by the responses). Senescent rats placed in a cold room became almost twice as hypothermic as the other groups. The deleterious effect of a cold environment has previously been shown in old mice and rats. Thirty month old mice have also been shown to be unable to maintain body temperatures during a 3 hour exposure to similar temperatures (Finch, Foster and Mirsky, 1969). During a 3 week exposure to 5 °C, only 36 percent of 25 month old Sprague-Dawley rats survived, but 100 percent of 3 to 4

month old survived in the same environment (Kiang-Ulrich and Horvath, 1979).

Several previously reported age-related differences could have been responsible for the reduced ability of senescent rodents to survive cold stress. Senescent rats have been shown to be relatively hypothyroid with diminished circulating levels of both thyroxine and triiodothyronine (Klug and Adelman, 1979 ; Sartin, Pritchett and Marple, 1977). It has been suggested that the thyroid is less responsive in senescent rats, since similar amounts of thyrotropin (TSH) were released in both young and old rats in response to cold but in old rats did not cause a rise in thyroxine (Huang, Steger and Meites, 1980). Hypothyroid rats were shown to be less responsive to beta-adrenergic receptor agonists (Fregly et al., 1975) and therefore catecholamine functions in thermoregulation may be affected. Although corticosterone levels in senescent Fischer 344 rats were similar to those in young rats, the adaptive increase in corticosterone levels in response to a stress (starvation) was greatly reduced in senescent rats (Adelman et al., 1978).

One would expect that during acute exposure to the cold, shivering thermogenesis and peripheral vasoconstriction would predominate as defenses. There have been no studies of shivering thermogenesis in aged rodents or man. The inability of senescent rats to maintain body temperature as well as young rats did in this study was probably due to impaired ability to activate heat production and prevent heat loss. It is not surprising that older, less

metabolically active rats would have a decreased capacity to respond and less reserve when heat production and inhibition of heat loss are fully challenged. Rats of any age immobilized by restraint are evidently not able to maintain body temperatures as well as non-restrained rats because of interference with behavioral thermoregulatory acts such as assuming a compact posture (McDougal, Marques and Burks, submitted).

To test the hypothesis that senescent rats were also more susceptible to heat stress than young rats, the rats were exposed to a warm humid environment. Rats restrained in this environment were also unable to keep body temperatures from rising, due in part to interference of restraint with behavioral thermoregulatory acts such as saliva spreading and sprawling. Others have shown that young and mature female rats avoided an increase in body temperature at 31 ^{o}C when allowed to move freely (Romelspacher, Schulze and Bolt, 1975). Evaporative heat loss through respiration can be an important means of heat loss in the rodent, therefore the lower respiratory rate of the senescent rats may have been responsible for their increased hyperthermia compared to young rats. However, the relative humidity of the warm room was approximately 60 per cent and this condition would be expected to allow much less heat loss due to evaporation than a warm dry environment. Exposure to cold and heat stresses suggested that without the benefit of behavioral thermoregulation, which was denied by restraint, senescent rats were less able to compensate for thermal stress than young rats. It is possible that

old rats are more dependent on behavioral thermoregulation than young rats, however comparisons of the effects of the warm and cold stresses in unrestrained rats would be necessary to determine if the importance of behavioral thermoregulation increases with age.

Similar to those reported in the literature (Ary and Lomax, 1979 ; Burks and Rosenfeld, 1979a), the effects of morphine on rectal temperature depended on dose and route of administration. In studies with other rats, which would be classified as mature in this study, Spratto and Dorio (1978) suggested age-related differences similar to those found here. They reported that a hypothermic dose of morphine (50 mg/kg i.p.) caused less hypothermia in 10 month old rats than it did in 1.5 month old rats. In the present study, hypothermia induced by the two highest doses of subcutaneous morphine was dramatically less in the senescent rats. Spratto and Dorio also found that a hyperthermic dose caused the least hyperthermia in their youngest group. Similarly, the young rats' hyperthermic response to the low dose of morphine was less than the response of the senescent rats in the present study. However, despite statistical significance, due to the small size of the hyperthermic responses in all groups the differences were probably not as physiologically meaningful as the differences in hypothermia. In contrast to subcutaneous morphine, an age-related difference in the responses to intravenous morphine, as measured by integrated response, was not present.

The reasons for the age-related differences with subcutaneous but not intravenous administration of morphine are elusive and a caveat about interpretation is appropriate. The thermic effects of intravenous morphine were characterized at only one dose and at that dose the maximum hypothermic response showed age-related differences but the integrated hypothermic response did not. Whether the effect of intravenous morphine differs with aging could be argued either way. Analgesia due to intravenous morphine also has a tendency to be greater in the young rats and therefore does not clarify the situation. Decreases in peripheral blood flow (Bender, 1965) could possibly have caused slower or less complete absorption of morphine from subcutaneous sites in senescent rats. However, the subcutaneous absorption of tritiated water, which should directly relate to blood flow, did not show an age-related difference In addition, an intraperitoneal injection (Spratto and Dorio, 1978) produced age-related thermic responses similar to subcutaneous responses. Therefore it is assumed that a decreased subcutaneous blood flow is not responsible for the age-related differences in the effect of subcutaneous morphine. It is possible however, that some other age-related change which would affect the absorption of morphine but not tritiated water is responsible for a difference in the subcutaneous response. Subcutaneous absorption of radiolabeled morphine would be a means of ruling out this possibility.

One method frequently used to determine the effect of absorption and distribution is to administer a drug directly at a

site of action. When this was accomplished with morphine, injections directly into the brain resulted in hypothermia at all doses. This response was in contrast to the biphasic response (hyperthermia at low doses and hypothermia at high doses) produced with subcutaneous morphine. When morphine was administered directly into the lateral ventricle, age-related differences occurred only at the highest doses. At these high doses senescent rats were more hypothermic than the young rats. Only at the two highest doses of morphine (150 and 200 ug) was the hypothermia produced as great as that produced by subcutaneous morphine (25 mg/kg). The age-related differences were in the opposite direction compared to subcutaneous morphine. This might suggest differences in blood-brain barrier permeability, but it has been reported that there is not an age-related change in the blood-brain barrier as measured by cerebrovascular permeability to intravenously administered ^{14}C-sucrose (Rapoport, Ohno and Pettigrew, 1979). A scanning electron microscope comparison of the floor and lower walls of the third cerebral ventricle in senescent and young rats showed structural changes with age (Scott and Sladek, 1981). Tanycytes, which are essential to the integrity of the blood-brain barrier, were shown to be farther apart in senescent rats. It is most likely that the increased hypothermic responses in the old rats with i.c.v. administration is due to greater access of morphine to the hypothalamus via the floor of the third ventricle. Interestingly, the time for maximal effect of morphine appears to be slightly longer with the i.c.v. route of administration than with

subcutaneous administration. This suggests that morphine injected into the lateral ventricle must diffuse to its site of action.

In an effort to determine the CNS changes which were responsible for the greater sensitivity of the senescent rats to the hypothermic effects of i.c.v. morphine, the actual putative neurotransmitter (rather than synthetic agonists) involved in thermoregulation were injected into the lateral ventricle to test for age-related differences. Doses of norepinephrine, acetylcholine and dopamine were chosen which caused a downward shift of rectal temperature of approximately 2 ^{o}C. At these doses norepinephrine and dopamine did not cause any age-related differences in thermic response. Acetylcholine, however, caused less hypothermia in the young rats than it did in the mature and senescent rats. Several age-related changes in cholinergic sensitivity have been reported. Microiontophoretically applied acetylcholine was shown to stimulate the firing of hippocampal pyramidal cells much less effectively in aged than in young rats (Lippa eta al., 1980). Changes in brain cholinergic mechanisms have been suggested to be responsible for memory loss observed in aged subjects because scopolamine, a central cholinergic blocker, causes memory impairment in young normals (Drachman, 1977 ; Drachman and Leavitt, 1974), and physostigmine can improve memory in the impaired elderly (Davis, Mohs and Tinklenberg, 1979).

The age-related differences in cholinergic response discovered here were exciting for several reasons. First, no age-related differences in thermic response to cholinergic agonists have been previously reported. Second, differences in cholinergic thermic response support and extend the age-related cholinergic differences discussed above. Acetylcholine has been shown to increase heat loss whether injected into the ventricle or POAH of rats (see review by Crawshaw, 1979). Rats have been shown to exhibit both behavioral (Cox, Green and Lomax, 1975) and physiological (Meeter, 1971) heat loss responses to cholinergic agonists. Third, the similarities between the age-related differences in i.c.v. opiate and cholinergic responses (senescent rats became more hypothermic in both instances) are consistent with the idea that the hypothermic response to i.c.v. morphine is partly mediated via a cholinergic heat loss mechanism.

Morphine administered orally did not show age-related differences in thermic response. Morphine intubated into the stomach caused only hyperthermia, the onset of which was directly related to dose. The most likely explanation for a hyperthermic response to oral morphine when given in high doses is that the thermoregulatory centers are only exposed to a low dose of morphine (which causes hyperthermia). Morphine has been shown to interfere with its own absorption after oral administration as determined by analgesic effect and blood levels (McDougal, Butler and Burks, 1981). The lack of age-related differences with the oral route of administration is not entirely consistent with the idea that only a low dose reaches

thermoregulatory centers e.g. with a low dose of morphine the senescent rats were more hyperthermic than the young rats after the first 1.5 hours, but with oral morphine a similar age-related difference was not found. However, only slight age-related differences in subcutaneous absorption or gastric absorption of morphine could offset similarities between routes of administration.

There were several indications that the age-related differences in thermic response to acute morphine were due to the effects of morphine rather than non-specific changes in the thermoregulatory system. Predominant among these were the responses to the general anesthetic effect of ethanol. Neither 1.5 nor 3 g/kg of ethanol caused a difference in the extent of hypothermia. Ethanol in both doses caused unconsciousness and inhibition of thermoregulation, and responses of the age groups were identical regardless of the dose. In addition, no age-related differences in response to norepinephrine or dopamine in naive rats were found. If the different responses in the senescent rats were due to general deficits in the thermoregulatory system, such as inability to lose heat, one would expect differences to be in a consistent direction.

Several groups have investigated opiate binding sites for age-related changes. Hess, Joseph and Roth (1981) showed that the binding site for ^{3}H-etorphine in rat striatum, frontal poles and hippocampus decreased in number with aging. Binding sites in the cortex and amygdala also decreased but not significantly. Messing and associates (1980) found decreased density of ^{3}H-dihydromorphine

binding in thalamus and midbrain of aged rats. Pedigo and McDougal (unpublished results) found decreased ^{3}H-naloxone binding sites in the hypothalamus and corpus striatum of old rats. Several problems are inherent in the interpretation of these studies. First, all studies showed a tendency for binding site numbers to decrease with aging and all groups expressed binding per milligram protein. Senescent rat brains observed in this study were noticably more white and fatty appearing. It is therefore possible that by expressing binding per milligram protein that a decrease in binding sites is artificially induced. Another problem acknowledged by Hess, Joseph and Roth (1980) was the lack of correlation between binding site concentrations in any brain region with sensitivity to pain. Additionally, they found that there was no correlation between binding site levels in various brain regions. For these and other methodological problems inherent in binding studies, it cannot be determined if opiate binding site changes are responsible for age-related differences in response.

It was unusual that age-related differences in response to morphine were present with some routes of administration but not others, however considering the diverse effects of morphine in various routes of administration, it was not contradictory. Morphine administered orally caused only hyperthermia, but morphine administered intracerebroventricularly caused only hypothermia. Subcutaneously administered morphine caused a hypothermia followed by a hyperthermia, the magnitudes of which depended on dose. These

differences due to route of administration can be explained by two sites of action of morphine.

A two site model for the thermoregulatory effects of morphine (Figure 44) encompases one site for the hyperthermia and one site for the hypothermia. These sites are anatomically and functionally separate and appear to have different thresholds for activation. Activation of the hypothermic site by morphine probably results in increased heat loss and decreased heat production, whereas activation of the hyperthermic site results in decreased heat loss or increased heat production. Activation of the hypothermic site causes a decrease in temperature of up to 6 ^{o}C, but activation of the hyperthermic site causes an elevation of temperature of only 2 ^{o}C.

The i.c.v. results (hypothermia at any dose) suggest that only a hypothermic site is accesible via the ventricle, whereas Cox, Chesarek and Lomax, (1976) have shown that discrete injections of morphine into the POAH can cause hyperthermia. Morphine given s.c. seems to be able to reach both sites as evidenced by biphasic responses, however, at low doses only the hyperthermic site may be reached. From these studies it is not known if intravenous morphine causes a similar biphasic response, but from the model it would be predicted to be biphasic i.e. activate both sites. Oral morphine appears to only have the capability to activate the hyperthermic site probably because the threshold for hypothermia is not reached due to inhibition of absorption. Rudy and Yaksh (1977) reported that morphine administered intrathecally caused only hyperthermia, and it

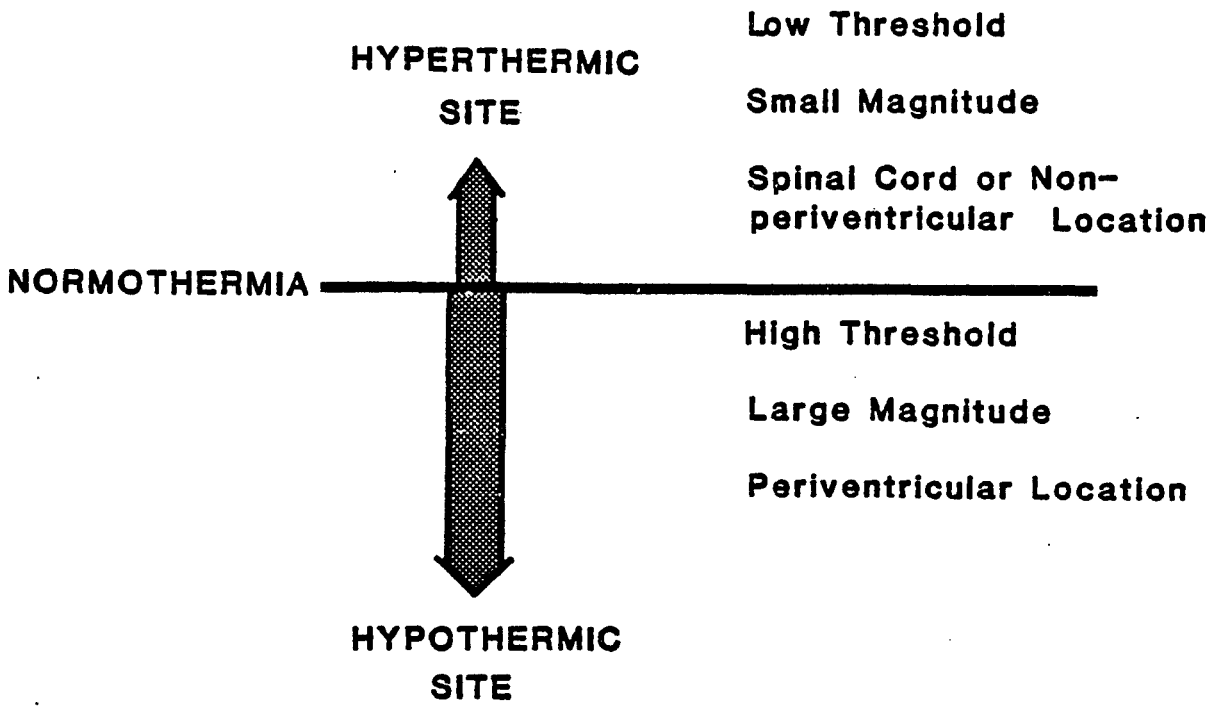

Figure 44. Two site model for the thermic effects of morphine. Arrows represent distinct sites with opposite effects on body temperature.

is entirely possible that the cord is the important hyperthermic site which is reached by subcutaneous and oral morphine but not by morphine given i.c.v.

Complicating the interpretation of studies of this type is the fact that the responses of interest (hyperthermia and hypothermia) are opposing and the actual response observed is probably an algebraic sum of both responses. For example, subcutaneous morphine responses could be explained by a hyperthermic site which has a low threshold and a hypothermic site which has a high threshold for activation. Below the threshold for activation of the hypothermic site, hyperthermia is the response. Just above the hypothermic threshold, sequential responses or the algebraic sum of responses could be expected. When hypothermia is fully activated, the hyperthermic response could be fully counteracted and the only apparent response would be hypothermia. Complicating the matter more is inertia in the thermoregulatory system which could blunt and delay responses.

Another possibility is that the rate of access of morphine to central receptors with various routes of administration may have been a determinant of the response. A rapid rate of activation of opiate receptors could have resulted in a tachyphylaxis which resulted in less of a response. As support, the integrated hypothermic response to intravenous morphine was less than the response to subcutaneous morphine.

Age-related differences in the thermic responses to morphine can be explained by changes in the hyperthermic site. With subcutaneous morphine (proposed to activate both sites), the young rats became significantly more hypothermic and the senescent rats were slightly more hyperthermic. There was a small age-related difference with i.c.v. morphine (proposed to activate hypothermic site), but only with very high doses which also caused vocalization and violent behaviors. These results are consistent with the idea that the hyperthermic site in the senescent rats is more sensitive or capable of stimulation by morphine.

Hess, Joseph and Roth (1981) have reported a slight increase in heat perception latency (tail-flick) with aging after studying 70 Wistar rats. In the study reported here no differences in baseline thermal pain sensitivity were found. Similar to the thermic effect of i.v. morphine, the inhibition of tail-flick latency with i.v. morphine did not differ between the age-groups. With subcutaneous morphine, the young rats appeared to be more sensitive to the analgesic as well as the thermic effects of morphine. The analgesic responses to morphine are very similar to the thermic responses after s.c. administration, e.g. young rats are more sensitive to s.c. morphine with both tests. These results are similar to those of Spratto and Dorio (1978) who found that 1.5 month old rats were more sensitive than 10 month old rats to the analgesic effects as measured by tail flick. These results also agree with the increased sensitivity to morphine-induced increases in tail-flick latency of 3

month old mice compared to 28 month old mice (Webster, Schuster and Eleftherio, 1976). Thus the results in rodents are in contrast to the verbal reports of increased pain relief due to morphine in aged patients (Bellville et al., 1971 ; Kaiko, 1980). It would not be easy to determine whether these differences between rodents and humans are due to innate differences in the perception of pain with aging or due to the emotional nature of pain perception in humans. However, these studies may not be comparable because of the differing subjective response to pain.

Studies of aged rodents have shown that the sensitivity to the lethal respiratory depressant effects of morphine increases with age (Chen and Robbins, 1943 ; Spratto and Dorio, 1978). This study suggests that morphine administered intravenously depresses respiration to the same extent in all age groups; however, due to the physiological changes in the respiratory system with aging, morphine is more lethal in the senescent rodent. The 25 percent decrease in baseline respiratory rate with aging and an increased arterial PCO_2 and decreased arterial $_pH$ (Rapoport, Ohno and Pettigrew, 1979) suggest that the respiratory system of the aged rodent is more susceptible to the lethal effects of morphine. Humans also show age-related decreases in function of the respiratory system, especially decreases in arterial PO_2 (Murray, 1976) and decrease in sensitivity of peripheral chemoreceptors to changes in arterial PO_2 and PCO_2 (Kronenberg and Drage, 1973). The limiting factor in the use of high doses of morphine for pain relief is lethality due to respiratory

depression, and it is possible that aged humans could be more at risk with high doses of morphine.

Morphine administration subcutaneously or i.c.v. in a single dose was capable of causing tolerance as reported previously (Gunne, 1960; Lotti, Lomax and George, 1966 ; Rosenfeld and Burks, 1977). Interestingly, morphine tolerance was acquired less readily in the senescent rats compared to the young rats. Senescent rats did not acquire tolerance to the low dose of subcutaneous morphine although the other rats did. Senescent rats became tolerant with higher doses and pellet implantation; however, the degree of tolerance as measured by integrated responses, was less. Tolerance to drugs is a homeostatic response to the effects of that drug. Although the specific mechanisms differ depending on the drug induced imbalance, tolerance to a drug is apparent when, due to compensatory mechanisms, the drug produces less of a response with subsequent administration. In other words, the organism adapts to the effects of a drug. Aging has been described as a decrease in adaptability (Adelman, 1979) and tolerance can be considered adaptation to drugs.

Another interesting explanation for the slower acquisition of tolerance in the senescent group depends on the fact that subcutaneous morphine caused less hypothermia in senescent than young rats. The homeostatic response to a drug (tolerance) would be expected to be proportional to the drug effect, and since hypothermia was caused by morphine in the senescent rats, one would expect the homeostatic compensation to be less. The results of tolerance with

i.c.v. morphine do not support this notion, since in this case, tolerance in senescent rats was not as complete but the hypothermic response to i.c.v. morphine was greater. The most likely explanation, therefore, is decreased adaptability. This decreased adaptability is also supported by heat and cold stress data. If aged humans are also more resistant to morphine tolerance, it may be possible to take therapeutic advantage of this decrease in adaptability.

All age groups exhibited morphine tolerance after implantation of pellets for 72 hours, as assessed by a morphine challenge and naloxone-precipitated withdrawal. In order to determine mechanisms of the differences in tolerance, cross-tolerance of putative transmitters with morphine was investigation. The sensitivity of transmitters involved in thermoregulation would be expected to change when a drug that affects thermoregulation, such as morphine, is continuously administered.

Age-related differences in cross-tolerance to morphine were found with norepinephrine, dopamine and acetylcholine. Morphine-tolerant senescent rats showed cross-tolerance (subsensitivity) with norepinephrine and dopamine, but the young rats did not. Young rats showed cross-tolerance (subsensitivity) with acetylcholine, but senescent rats did not. These changes may suggest a differential sensitivity of post synaptic elements to endogenously released transmitters due to development of tolerance. These subsensitivities induced by morphine tolerance reflect a shift from catecholaminergic

to cholinergic systems with aging as a means of homeostatic compensation for morphine-induced decrease in heat production and increase in heat loss.

In summary, senescent rats respond to morphine differently than young rats. The differences in response are due to both quantitative (decreased adaptability and reserve) and qualitative (shift in transmitter systems) differences inherent in the aging process. Whether the rat is a good model for opiate responses or thermoregulation in aged humans can not be precisely determined, because opiate effects on thermoregulatory functions in man have not been studied in depth. The rat model has the potential to help with the understanding of geriatric pharmacology. Studies on senescent rodents have been criticized for utilizing the survivors, or the healthy elderly population, but unhealthy elderly humans, as well as, unhealthy elderly rats do not survive. By their nature, many geriatric clinics see a larger proportion of the ill aged population than the healthy aged, however, an aging study which ignores the healthy senescent population would be of limited value to those who would like to understand the normal aging process.

CONCLUSIONS

1. Senescent rats thermoregulate at lower "normal" body temperatures than young rats and are less able to maintain body temperature in warm or cold environments.

2. Senescent rats are less responsive to the hypothermic and analgesic effects of subcutaneous morphine, but not because of reduced blood flow at the site of injection due to aging.

3. The age-related differences in response to morphine were not due to inherent differences in the thermoregulatory system, since hypothermia was increased, decreased or not changed, depending on treatment.

4. Previously reported increased lethality of intravenous morphine in aged rodents is not due to increased sensitivity to the respiratory depressant effects of morphine but to decreased respiratory reserve.

5. Tolerance to morphine was acquired less readily in the senescent rats than in the young rats, reflecting decreased adaptability.

6. Cross-tolerance of neurotransmitters with morphine suggests a shift from catecholaminergic to cholinergic systems with aging as a means of homeostatic compensation for morphine's thermic effects.

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