

IN VITRO CHARACTERIZATION OF ADRENERGIC  
RECEPTORS CONTROLLING SKIN GLAND  
SECRETION IN TWO ANURANS  
RANA PIPIENS AND XENOPUS LAEVIS

by

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## ABSTRACT

Catecholamines stimulate secretion from granular glands of the amphibian integument. The in vitro response (secretion) of the integumental granular glands of Rana pipiens and Xenopus laevis to sympathomimetic stimulation was studied in order to characterize the nature of the adrenergic receptors possibly controlling this response. The in vitro response of skin glands to the following sympathomimetic agents was studied: epinephrine (E), norepinephrine (NE), phenylephrine (PE), and isoproterenol (ISO). Epinephrine and norepinephrine caused secretion from the granular glands, whereas phenylephrine was effective in causing secretion only at higher concentrations. Isoproterenol could not elicit secretion at any concentration used. These agonists could be ranked in the following order of relative effectiveness: E and NE > PE > ISO. This is the classical ranking for an alpha adrenergic receptor mediated response. The alpha adrenergic antagonists, Dibenamine, phentolamine, and ergotamine blocked the response of skins to NE and E. In contrast, this response was not blocked by either dichloroisoproterenol (DCI) or propranolol, both potent beta adrenergic blocking agents.

Smooth muscles envelop the granular glands of amphibians. The methylxanthine, theophylline, a smooth muscle relaxant, blocked the response of granular glands to the alpha agonists in both frogs. Granular gland secretion in response to catecholamines is mediated by smooth muscle contraction resulting from alpha adrenergic stimulation.

## CHAPTER I

### INTRODUCTION

Skin glands are common to the integument of most amphibians. These glands are of two types, mucous and granular (poison, serous; Noble, 1931). Surrounding the granular glands is a layer of smooth muscle, the contraction of which apparently results in glandular secretion. Injected catecholamines are known to stimulate secretion from these glands (in Xenopus laevis; Burgers, 1956: in Rana pipiens; Bastian and Zarrow, 1954). At present it is not known whether the source of endogenous catecholamine control derives from adrenomedullary sources or from adrenergic nerve terminals directly innervating these glands. Although granular gland secretion may be stimulated by catecholamines, there is at present little or no definitive information as to the nature of adrenergic receptors possibly mediating this response. Recently, Watlington (1968) has suggested that secretion from mucous glands may be regulated by beta adrenergic receptors. The present study was undertaken to determine the nature of the adrenergic receptors that may control secretion of the granular glands.

## CHAPTER II

### METHODS AND MATERIALS

Two species of anurans were used in this study. The leopard frog, Rana pipiens, was obtained from the Lemberger Company, Oshkosh, Wisconsin. Both male and female adult specimens were utilized in approximately equal numbers in each experiment. South African clawed toads, Xenopus laevis, were obtained from South Africa. Only females of this species were utilized in the experiments described here.

Animals were sacrificed by decapitation followed by spinal pithing. Dorsal (back), ventral (abdominal), and leg skins were utilized in our experiments with X. laevis because of the apparent uniform distribution of skin glands in all these integumental areas. Only the back skin of R. pipiens was used because of the predominance of granular glands located within the dorsal plicae. After removal, skins were immersed immediately in amphibian Ringer solution (pH 7.4), and were then cut into smaller pieces, usually four or five in number. Each piece of skin was then placed in a 50 ml beaker containing 20 ml of Ringer solution. Skins were usually allowed

to reside in the Ringer solution for approximately 30 minutes before addition of hormonal or pharmacological agents.

To characterize the nature of possible adrenergic receptors mediating glandular secretion from the skin of X. laevis and R. pipiens, the "l" isomers of the following sympathomimetic agents were used: epinephrine (E), norepinephrine (NE), phenylephrine (PE), and isoproterenol (ISO). These agents were used as bitartrates except PE which was obtainable only as a hydrochloride. The drugs were obtained from the Winthrop Laboratories (Special Chemicals Department). The following alpha adrenergic blocking agents, Dibenamine hydrochloride (K and K Laboratories), phentolamine hydrochloride (CIBA Pharmaceutical Company), and ergotamine tartrate (Sigma Chemical Company) were used as antagonists to sympathomimetic stimulation. The beta adrenergic blocking agents, dichloroisoproterenol (DCI), and propranolol, both obtained from Aldrich Chemical Company, were similarly utilized. The methylxanthine, theophylline (Nutritional Biochemical Company), was used as a smooth muscle relaxant (Ritchie, 1966). Except for theophylline, the hormonal and pharmacological agents were added in 0.2 ml amounts to the 20 ml of Ringer solution bathing the skins. Due to the low solubility of the methylxanthine, Ringer solution containing this agent was

substituted for the original Ringer solution in which the skins had been placed after excision. All concentrations of the agents used in these experiments are expressed as the final molar concentrations of the agents after addition to the skins.

Skin gland responses to sympathomimetic stimulation were evaluated under a dissecting microscope. The presence of a white exudate from the glands was considered a positive (+) response, while the absence of such a secretion was monitored as a negative (0) response. In all cases, positive responses were only considered valid when the skins remaining in Ringer solution, as controls, did not themselves reveal secretion. In those experiments involving the use of adrenergic blocking agents, blockade was only considered valid when other Ringer control skins from the same animal were able to secrete in response to catecholamine stimulation.

## CHAPTER III

### RESULTS

#### Sympathomimetic Stimulation

The first experiments were designed to evaluate the response of granular glands to sympathomimetic stimulation. Three X. laevis were sacrificed and their skins subjected to the following sympathomimetic amines: epinephrine (E), norepinephrine (NE), phenylephrine (PE), and isoproterenol (ISO). In Table 1 are shown the results of these experiments. Whereas ISO was ineffective in stimulating secretion at any concentration used, both NE and E were effective even at fairly low concentrations. Phenylephrine elicited glandular secretion only when higher concentrations were used. The sympathomimetic agents could therefore be ranked as to their ability to evoke glandular secretion in the following order of relative effectiveness: E and NE > PE > ISO. The glands of the dorsal skin had a much lower threshold to stimulation than did those of the ventral skin.

Similar experiments with R. pipiens gave comparable results. Both E and NE caused secretion; again, PE was only effective at the higher concentration used (and then, not all skins responded) and ISO was totally ineffective

Table 1. Response of Integumental Glands of Xenopus laevis to Sympathomimetic Stimulation

Three toads each supplied five pieces of skin, four of which were subjected to a low concentration ( $10^{-5}$  M) of the sympathomimetic agents, one piece of skin for one agent. The fifth or remaining piece of skin was maintained in Ringer solution as a control (A). Those skins that did not respond at this concentration were then subjected to a higher concentration ( $10^{-4}$  M) of these agents (B).

	Frog Number	Ringer Solution	Phenylephrine	Norepinephrine	Epinephrine	Isoproterenol
A.	1	0	0	+	+	0
	2	0	0	+	+	0
	3	0	0	+	+	0
Total	3	0	0	3	3	0
B.	1		+			0
	2		+			0
	3		+			0
Total	3		3			0

(Table 2). Thus, the sympathomimetic agents could again be similarly ranked as to their ability to induce glandular secretion in this second species of anuran. As is evident from the totals, the glands of some skins were not reactive at the concentration used in these experiments.

#### Effect of Adrenergic Antagonism

Although the characterization of adrenergic receptors based on their physiological response to agonists was demonstrated, one can more clearly differentiate between alpha and beta receptor mediated responses by the use of adrenergic antagonists. Therefore, in the next experiment, 18 R. pipiens were sacrificed and part of their skins were preincubated in ergotamine. After one hour preincubation in this alpha adrenergic blocking agent, NE ( $10^{-4}$  M) was added to the skins. Norepinephrine induced secretion from those skins residing in Ringer solution (control response), but the alpha adrenergic blocking agent, ergotamine, blocked the response of granular glands to norepinephrine stimulation (Table 3).

A similar experiment utilizing Dibenamine as the alpha adrenergic antagonist provided analogous results to those obtained in Table 3. Twelve R. pipiens were sacrificed and a portion of their skins were preincubated in Dibenamine ( $10^{-4}$  M) while the remaining pieces of skin were maintained in Ringer solution. After one hour, NE ( $10^{-4}$  M) was added

Table 2. Response of Integumental Glands of Rana pipiens to Sympathomimetic Stimulation

Twenty-seven frogs each supplied five pieces of skin, one each of which was subjected to a  $10^{-4}$  M concentration of one of the sympathomimetic agents. One skin from each frog was maintained in Ringer solution as a control.

Frog Number	Ringer Solution	Phenylephrine	Norepinephrine	Epinephrine	Isoproterenol
1	0	0	+	+	0
2	0	0	+	+	0
3	0	0	+	+	0
4	0	0	+	+	0
5	0	0	+	+	0
6	0	0	+	+	0
7	0	0	+	+	0
8	0	0	+	+	0
9	0	0	+	+	0
10	0	+	0	+	0
11	0	0	+	0	0
12	0	+	+	+	0
13	0	0	+	+	0
14	0	0	0	+	0
15	0	0	+	0	0
16	0	0	0	+	0
17	0	0	+	+	0
18	0	0	+	+	0
19	0	0	+	+	0
20	0	0	+	+	0
21	0	0	+	+	0
22	0	0	+	+	0

Table 2 (Continued).

Frog Number	Ringer Solution	Phenylephrine	Norepinephrine	Epinephrine	Isoproterenol
23	0	0	+	+	0
24	0	0	+	+	0
25	0	0	+	+	0
26	0	0	+	+	0
27	0	0	+	+	0
Total	27	0	24	25	0

Table 3. Blockade of Sympathomimetic Induced Skin Gland Secretion by Ergotamine in Rana pipiens

Eighteen frogs each supplied four pieces of skin, two of which were incubated in ergotamine ( $10^{-4}$  M) and two pieces from each animal were untreated and were maintained in Ringer solution. After one hour NE ( $10^{-4}$  M) was added to one group of skins incubated in ergotamine and to one group of skins residing in Ringer solution. The remaining two groups of skins were maintained as controls.

Frog Number	Ringer Solution	Norepinephrine	Ergotamine	Ergotamine & Norepinephrine
1	0	+	0	0
2	0	+	0	0
3	0	+	0	0
4	0	+	0	0
5	0	+	0	0
6	0	+	0	0
7	0	+	0	0
8	0	+	0	0
9	0	+	0	0
10	0	+	0	0
11	0	+	0	0
12	0	+	0	0
13	0	+	0	0
14	0	+	0	0
15	0	+	0	0
16	0	+	0	0
17	0	+	0	0
18	0	+	0	0
Total	18	0	18	0

to the skins. Again, NE induced secretion from the glands of those skins residing only in Ringer solution; this response was blocked in those skins preincubated in Dibenzamine (Table 4).

One further experiment was done utilizing the alpha adrenergic antagonist, phentolamine, and similar results were obtained to those in Table 3 and 4. Fourteen R. pipiens were sacrificed and part of their skins were preincubated in phentolamine ( $10^{-4}$  M) while the remaining portions of skins were allowed to reside in Ringer solution during the incubation period. After one hour, NE ( $10^{-4}$  M) was added to the skins. Again, complete secretion was observed from granular glands in those skins maintained in Ringer solution while phentolamine totally blocked this secretory response in those skins which were preincubated in this agent (Table 5).

The next experiments were designed to determine the effect of the beta adrenergic receptors, if any are present. Therefore, six X. laevis were sacrificed and their skins preincubated in either propranolol or DCI (both potent beta adrenergic antagonists) and phentolamine. After one hour preincubation in these blocking agents, E ( $10^{-4}$  M) was added to the skins. Epinephrine induced secretion from those skins residing in Ringer solution (control response) as well as from those skins incubated in the

Table 4. Blockade of Sympathomimetic Induced Skin Gland Secretion by Dibenamine in Rana pipiens

Twelve frogs each supplied four pieces of skin, two of which were incubated in Dibenamine ( $10^{-4}$  M) and two pieces from each animal were untreated and maintained in Ringer solution. After one hour, NE ( $10^{-4}$  M) was added to one group of skins incubated in Dibenamine and to one group of skins residing in Ringer solution. The remaining two groups of skins were maintained as controls.

Frog Number	Ringer Solution	Norepinephrine	Dibenamine	Dibenamine & Norepinephrine	
1	0	+	0	0	
2	0	+	0	0	
3	0	+	0	0	
4	0	+	0	0	
5	0	+	0	0	
6	0	+	0	0	
7	0	+	0	0	
8	0	+	0	0	
9	0	+	0	0	
10	0	+	0	+	
11	0	+	0	0	
12	0	+	0	0	
Total	12	0	12	0	1

Table 5. Blockade of Sympathomimetic Induced Skin Gland Secretion by Phentolamine in Rana pipiens

Fourteen frogs each supplied four pieces of skin, two of which were incubated in phentolamine ( $10^{-4}$  M) and two pieces from each animal were untreated and maintained in Ringer solution. After one hour, NE ( $10^{-4}$  M) was added to one group of skins incubated in phentolamine and to one group of skins residing in Ringer solution. The remaining two groups of skins were maintained as controls.

Frog Number	Ringer Solution	Norepinephrine	Phentolamine	Norepinephrine & Phentolamine
1	0	+	0	0
2	0	+	0	0
3	0	+	0	0
4	0	+	0	0
5	0	+	0	0
6	0	+	0	0
7	0	+	0	0
8	0	+	0	0
9	0	+	0	0
10	0	+	0	0
11	0	+	0	0
12	0	+	0	0
13	0	+	0	0
14	0	+	0	0
Total	14	0	14	0

beta adrenergic blocking agents, DCI or propranolol. The alpha adrenergic blocking agent, phentolamine, in contrast, blocked the response of skins to E stimulation (Table 6).

Similar experiments with R. pipiens provided results comparable to those obtained with X. laevis. Here the alpha adrenergic blocking agent, phentolamine, again prevented glandular secretion in response to E stimulation (Table 7). Both propranolol and DCI failed to prevent glandular secretion in response to catecholamine stimulation.

#### Effect of Methylxanthines

Methylxanthines, e.g., theophylline and caffeine, are known to be smooth muscle relaxants and therefore were used to try and block the response of skin glands to catecholamine stimulation as the granular glands of anurans are encapsulated by smooth muscles (Noble, 1931). Therefore, in the next experiment, skins from R. pipiens were preincubated in theophylline for one hour and then NE was added. Whereas NE stimulated secretion from skins in Ringer solution (control response), those skins incubated in theophylline failed to secrete (Table 8).

Microscopical observations of cross sections of histological preparations of the dorsal plicae of R. pipiens skin previously successfully stimulated with E ( $5 \times 10^{-4}$  M)

Table 6. Failure of Beta Adrenergic Antagonists to Block Sympathomimetic Induced Skin Gland Secretion in Xenopus laevis

Six X. laevis each supplied five pieces of skin, one group of which was preincubated in the beta blocking agent DCI ( $10^{-4}$  M), another group was preincubated in another beta blocking agent, propranolol ( $10^{-4}$  M), and a third group of skins were preincubated in the alpha antagonist, phentolamine ( $10^{-4}$  M). The remaining two groups of skins were maintained in Ringer solution. After one hour, epinephrine ( $10^{-4}$  M) was added to one group of skins residing in Ringer solution and to the three groups preincubated in the adrenergic antagonists. The remaining group of skins in Ringer solution was untreated and served as a control for the experiment.

Frog Number	Ringer Solution	Epinephrine	DCI & Epinephrine	Propranolol & Epinephrine	Phentolamine & Epinephrine
1	0	+	+	+	0
2	0	+	+	+	0
3	0	+	+	+	0
4	0	+	+	+	0
5	0	+	+	+	0
6	0	+	+	+	0
Total	6	6	6	6	0

Table 7. Failure of Beta Adrenergic Antagonists to Block Sympathomimetic Induced Skin Gland Secretion in Rana pipiens

Twelve frogs each supplied five pieces of skin, one group of which was preincubated in the beta blocking agent DCI ( $10^{-4}$  M), another group was preincubated in another beta blocking agent, propranolol ( $10^{-4}$  M), and a third group of skins were preincubated in the alpha antagonist, phentolamine ( $10^{-4}$  M). The remaining two groups of skins were maintained in Ringer solution. After one hour, epinephrine ( $10^{-4}$  M) was added to one group of skins residing in Ringer solution and to the three groups preincubated in the adrenergic antagonists. The remaining group of skins in Ringer solution was untreated and served as a control for the experiment.

Frog Number	Ringer Solution	Epinephrine	DCI & Epinephrine	Propranolol & Epinephrine	Phentolamine & Epinephrine	
1	0	+	+	+	0	
2	0	+	+	+	0	
3	0	+	0	+	0	
4	0	+	+	+	0	
5	0	+	+	+	0	
6	0	+	+	+	0	
7	0	+	+	0	0	
8	0	+	+	+	0	
9	0	+	+	+	0	
10	0	+	+	+	0	
11	0	+	+	+	0	
12	0	+	0	0	0	
Total	12	0	12	10	10	0

Table 8. Effect of Theophylline on Skin Gland Responses to Sympathomimetic Stimulation in Rana pipiens

Ten frogs each supplied four pieces of skin, two pieces of which were allowed to remain in Ringer solution while the other two pieces were preincubated in theophylline ( $10^{-4}$  M). After one hour, NE ( $10^{-4}$  M) was added to one of the Ringer and to one of the theophylline groups of skin. The remaining two groups of skins were maintained as controls.

Frog Number	Ringer Solution	Norepinephrine	Theophylline	Theophylline & Norepinephrine
1	0	+	0	0
2	0	+	0	0
3	0	+	0	0
4	0	+	0	0
5	0	+	0	0
6	0	+	0	0
7	0	+	0	0
8	0	+	0	0
9	0	+	0	0
10	0	+	0	0
Total	10	0	10	0

revealed the presence of many collapsed, empty granular glands with their surrounding sheath of smooth muscles in a contracted state, as compared to adjacent unstimulated control sections of skin from the same animal. The mucous glands of the stimulated skins, in contrast, appeared unaffected by catecholamine stimulation; in both experimental and control skins these glands contained a similar amount of stainable secretory material and these glands were not collapsed in either case.

Both our microscopical investigations of cross sections of histological preparations of the dorsal plicae of R. pipiens and the studies of Bovbjerg (1963) utilizing histological preparations of the dorsal plicae of this same species reveal no sheath of smooth muscle surrounding the mucous glands of this anuran. The observations that no sheath of smooth muscle encapsulates the mucous glands and that these glands are unresponsive to E at a high concentration ( $5 \times 10^{-4}$  M) of this catecholamine are in opposition to those findings of Bastian and Zarrow (1954). These workers, reporting results of in vitro experiments, claim to have observed mucous glands in R. pipiens surrounded by a smooth muscle sheath which appeared contracted, evidencing secretion, after E stimulation. The concentration of E utilized in the experiments by Bastian and Zarrow

was similar to the concentration of this agonist used in our experiments for microscopical investigations of histological preparations of the dorsal plicae of R. pipiens.

## CHAPTER IV

### DISCUSSION

These results have clearly demonstrated that the response of the granular glands of the skin of both Xenopus laevis and Rana pipiens are responsive to sympathomimetic stimulation. Agonistic ranking studies have established that the relative effectiveness of sympathomimetic agents in inducing glandular secretion could be ranked in the following order: E and NE > PE > ISO. Such a ranking is characteristic of an alpha adrenergic receptor mediated response. This is further confirmed by the observations that PE, a specific, but weak, alpha agonist, induces granular gland secretion in both species, but that ISO, a specific beta agonist, is without such effect.

In addition, the fact that the alpha adrenergic blocking agents, Dibenamine, phentolamine and ergotamine, are effective in blocking the response of skin glands to sympathomimetic stimulation, while the beta adrenergic antagonists, dichloroisoproterenol and propranolol, are without such effect, is further evidence that secretion is mediated through alpha adrenergic receptors. These data also reveal that beta adrenergic receptors are not similarly involved in regulating this same response.

As it has been demonstrated by a number of workers that the granular glands of amphibians are encapsulated by smooth muscle, it is quite likely, therefore, that the alpha adrenergic receptors that subserve the secretory response are possessed by these smooth muscles. The fact that the methyloxanthine, theophylline, known to relax smooth muscle (Ritchie, 1966) blocks the response of these glands to sympathomimetic stimulation lends further support to such a suggestion. There is at present no definitive information regarding the normal source of catecholamines which stimulate secretion from the granular glands in vivo under conditions of stress.

Besides the granular glands normally present in the integument of amphibians, mucous glands are also found. These glands are not ensheathed by smooth muscles and there is at present little data as to the nature of the hormones or other agents that might be responsible for regulating the release of their mucous secretion, except for the report by Bastian and Zarrow (1954) proposing mucous gland secretion induced by E. These data are completely contrary to results of our experiments. In a recent communication by Watlington (1968) it was suggested that the response of mucous glands to sympathomimetic stimulation is mediated by beta adrenergic receptors. Our data, on the other hand, has shown that alpha adrenergic receptors regulate secretion

from granular (serous) glands. To our knowledge there has been no previous observations on the secretion of amphibian integumental mucous glands. It is possible, therefore, that the secretions observed by Watlington (1968) and by Bastian and Zarrow (1954) were from granular glands. Furthermore, it has been well documented (Koelle, 1966) that beta adrenergic receptors do not mediate the contractile response of smooth muscle but, rather, if they are present, then, they may relax the muscle if stimulated. In contrast, alpha adrenergic receptors, when present, are usually responsible for mediating smooth muscle contraction. The granular glands fit into this pattern whereby secretion is controlled by alpha adrenergic stimulation causing smooth muscle contraction. In addition, there is evidence (Lloyd, 1968) that alpha adrenergic receptors may regulate, in part, the control of mammalian sweat glands; these glands have been considered as being possibly homologous with the amphibian serous glands (Smith, 1960).

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