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Adrenergic Blocking Agents Modify Catecholamine Stimulation of Short-Circuit Current in Isolated Frog Skin (38086)

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Over the past two decades innumerable studies on sodium and chloride transport across living membranes have utilized the isolated short-circuited frog skin preparation of Ussing and Zerahn (1). The rate of transport of these ions has been correlated with Isc (1, 2). Early studies using this system showed that catecholamines stimulated sodium and chloride transport and Isc. More recently, the effect of catecholamine-blocking agents on catecholamine-stimulated sodium and chloride transport and Isc has come under scrutiny (3, 4). The present study is of the interactions of epinephrine (E), norepinephrine (NE), and isopropylarterenol (IPA) and the catecholamine-blocking agents phenoxybenzamine and propranolol on Isc. The data suggest that propranolol blocks catecholamine-stimulated Na^+ influx through isolated frog skin.

Materials and Methods. Female leopard frogs, *Rana pipiens,* from a northern supplier, were used in this experiment. They were received during February through April and kept unfed in stainless steel pans in flowing deionized water from a Continental Water Company deionizer. They were used after about 4–6 weeks in deionized water. The abdominal skin of each frog was divided into symmetrical halves, and each half was placed as a membrane separating two conical lucite chambers in an apparatus similar to that described by Ussing and Zerahn (1). Amphibian Ringer's solution (12.5 ml) was added to each side of the skin and circulated and aerated by moist air. Each liter of Ringer's solution used in these experiments contained NaCl, 109.4 m*M*; $NaHCO_3$, 2.8 m*M*; KCl, 1.88 m*M*; $CaCl_2$, 1.08 m*M*; and $MgCl_2 \cdot 6H_2O$, 0.5 m*M*. Since washout experiments showed that contact of the inner surface of the frog skin with catecholamine solutions for 1 min was sufficient to produce a maximum response lasting as much as 1 hr, we consider unimportant any breakdown of catecholamines which may have occurred in this bathing medium.

Short-circuit currents were recorded by the use of an automatic potential zeroing device, previously described, which maintains the skins continuously short-circuited while allowing short-circuit currents to be recorded (McAfee and Locke, 1967). The skins were allowed to stabilize for 1 hr before the drugs were added. The catecholamine-blocking agents used were propranolol (Inderal, Ayerst), at a concentration of 10 μM, and phenoxybenzamine hydrochloride (Dibenzylene, SK&F), at a concentration of 15 μM. The concentration of E (Adrenalin, Parke Davis), NE (Levophed, Winthrop), and IPA (Isuprel, Winthrop) were 10, 10, and 1 μM, respectively. All drugs were transferred from the vials in which they were supplied by the manufacturer into the bathing medium without delay. In paired skin experiments it was found that a concentration of 10 μM of E or NE produced a maximum response whereas less than 1 μM of IPA sufficed. Optimum blocking time for propranolol was determined in preliminary experiments; 20 min was required for maximum effect, but in the experiments reported here, 30-min pretreatment with propranolol was used.

TABLE I. The Effect of Propranolol (Prop) on the Increase in Isc of Isolated Frog Skin Produced by IPA, NE, and E. Description of Method in Text.

Experiment No.	N	Initial Isc in $\mu A/cm^2$ (mean ± SD)	Treatment	Increase in Isc $\mu A/cm^2$ skin (mean ± SD)	P
1	12	31.2 ± 11.1	IPA	30.7 ± 11.5	<.05
		30.2 ± 9.0	Prop + IPA	3.3 ± 4.0	
2	12	40.3 ± 7.5	NE	21.6 ± 8.1	<.05
		39.6 ± 7.3	Prop + NE	3.0 ± 2.3	
3	12	40.9 ± 13	E	14.4 ± 10	NS
		37.8 ± 11	Prop + E	11.1 ± 6.4	

Phenoxybenzamine had to be added to the solution 30 min prior to addition of the catecholamine to obtain consistent blockade of NE. The bathing medium with the blocking agent was not changed before the addition of catecholamines. All the substances used were added to the medium bathing the inside of the skin. Propranolol at the concentration used did not cause any change in the Isc by itself; however, phenoxybenzamine occasionally produced an increase in Isc which lasted less than 30 min. All experiments were paired. One half of a frog's abdominal skin was used to demonstrate the effect of the catecholamine alone, the other half was pretreated with propranolol or phenoxybenzamine. Means and standard deviations summarize the data and illustrate the degree of variability that is present between frogs with respect to their response to catecholamine. The Student's *t* distribution was used to test for the significance of the difference in the stimulation produced by the catecholamine alone and propranolol or phenoxybenzamine plus catecholamine.

Results and Discussion. Table I shows the effect of propranolol in blocking the stimulation produced by the catecholamines used in these experiments. Propranolol blocked the stimulation produced by IPA and NE, but it did not block the stimulation of Isc produced by E.

Table II contains the results of the experiments using phenoxybenzamine as a blocking agent. At the dosage used, phenoxybenzamine blocked the effect on Isc of all catecholamines tested.

Reports by other investigators indicate that the increased Isc produced by NE is the result of increased inward Na^+ transport (6). Similarly, we have reported that the increased Isc produced by IPA can be accounted for by an increased inward Na^+ transport (3). In contrast, the increase in Isc produced by E is thought to be due to outward Cl^- transport (2). Since propranolol inhibited the increase in the Isc produced by NE and IPA but did not inhibit the increase produced by E, it appears that catecholamine-stimulated inward Na^+ transport is inhibited by propranolol but catecholamine-stimulated outward Cl^- trans-

TABLE II. The Effect of Phenoxybenzamine (Phenox) on the Increase in Isc of Isolated Frog Skin Produced by IPA, NE, and E. Description of Method in Text.

Experiment No.	N	Initial Isc in $\mu A/cm^2$ (mean ± SD)	Treatment	Increase in Isc $\mu A/cm^2$ (mean ± SD)	P
1	10	24.6 ± 4.8	IPA	17.3 ± 5.7	<.05
		24.5 ± 9.1	Phenox + IPA	4.1 ± 4.1	
2	10	28.4 ± 6.6	NE	8.2 ± 3.0	<.05
		27.6 ± 8.5	Phenox + NE	0.3 ± 1.3	
3	10	37.0 ± 8.8	E	27.1 ± 11.8	<.05
		34.2 ± 15.8	Phenox + E	5.1 ± 4.5	

port is not. Our observations using phenoxybenzamine would seem to imply that both Na^+ influx and Cl^- outflux are inhibited by this material. Watlington (4) observed that phenoxybenzamine allowed an increase in Isc produced by E. The reason for the difference in our observations and his may be that we allowed 30 min for phenoxybenzamine to act while he allowed only 6 min. Our data are not readily explained using the now classical alpha and beta adrenergic receptor model. According to this model, propranolol should not block the action of NE whereas it did so. Furthermore, phenoxybenzamine should not have blocked the action of IPA whereas it did so. These observations raise a question as to the applicability of the alpha and beta receptor model to catecholamine-stimulated transport of Na and Cl by isolated frog skin. The applicability of the alpha and beta receptor concept has been questioned in relation to catecholamine effects on some other tissues (7–9).

Summary. An investigation is reported on the effects of phenoxybenzamine and propranolol on the catecholamine-stimulated short-circuited current (Isc) of isolated frog skin. Phenoxybenzamine and propranolol inhibited the increase in Isc produced by both norepinephrine (NE) and isopropylarterenol (IPA), whereas that produced by epinephrine (E) was inhibited by phenoxybenzamine but not by propranolol. We conclude that in the dosages of the drugs used, catecholamine-stimulated sodium influx through isolated frog skin is blocked by propranolol and phenoxybenzamine while chloride outflux is blocked by phenoxybenzamine but not by propranolol.

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