Studies on Adrenoceptors Involved in Regulation of Sodium Transport in Frog Skin

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ABSTRACT

To ascertain the existence of various adrenoceptors involved in active transport of sodium in the frog skin and to delineate their physiological roles, the influence of various adrenergic agonists and antagonists on the potential difference (PD), short-circuit current (SCC) and total skin conductance (TSC) of the isolated frog skin of Rana nigromaculata were investigated. PD and SCC were determined with Ussing's technique. Drugs were administered to the serosal side of the skin. Experimental results were summarized as follows:

1. The responses to norepinephrine (NE, $6 \times 10^{-8} - 6 \times 10^{-5}$M), phenylephrine (PE, $5 \times 10^{-6} - 5 \times 10^{-4}$M) and epinephrine (Epi, $5.5 \times 10^{-7} - 5.5 \times 10^{-5}$M) were characterized by marked elevation of PD & SCC in dose-related fashion, but the maximal effect attained by Epi was less than those of NE and PE.
2. These increments of PD & SCC were significantly inhibited by prazosin (2×10^{-6}M), a specific α_1-adrenoceptor blocker. The stimulatory effect on PD & SCC were completely abolished by phenoxybenzamine (PBZ, 3.3×10^{-5}M), an irreversible α-adrenoceptor blocking agent. Furthermore, with a larger doses of Epi produced marked decline of PD & SCC after the PBZ pretreatment.
3. Isoproterenol (ISP), a β-adrenoceptor agonist, in concentrations ranging from 5×10^{-7} to 5×10^{-6}M produced dose-related decrease in PD & SCC, which could be abolished by pretreatment with propranolol (4×10^{-6}M), a specific β-adrenoceptor blocker. It was further noted that the effects of Epi on PD & SCC were markedly potentiated by propranolol pretreatment.
4. Clonidine as well as guanabenz produced increases in PD & SCC and these effects were inhibited more specifically by prazosin pretreatment than by yohimbine.

These results indicated that there exist in the frog skin two distinctive types of adrenoceptors, α and β, which roughly corresponds to those in mammals, and that the α type of adrenoceptors mediate the stimulation of PD & SCC, whereas β-adrenoceptors mediate the inhibition. However, based on evidence at hand, no conclusion could be drawn on the subtype of α-adrenoceptors which is involved in the stimulation of sodium transport in the frog skin.

Key Words: Sodium transport in frog skin, adrenergic system, subtypes of alpha-adrenoceptor

Abbreviations: PD; potential difference, SCC; short-circuit current, TSC; total skin conductance, NE; norepinephrine, PE; phenylephrine, Epi; epinephrine, ISP; isoproterenol, PBZ; phenoxybenzamine

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INTRODUCTION

Huf (1935) first showed that frog skin bathed with Ringer's solution on both sides transports salt from the outside to the inside, and Krogh (1937) demonstrated that the skin of living frog was able to take up NaCl from a very dilute solution. Ussing (1949) reported that sodium transport is active, but chloride transfer was found to conform to the simple flux equation for an ion which moves passively through the skin. Thereafter, Ussing & Zerahn (1951) have shown that the short-circuit current measured in an external circuit, when the spontaneous potential of the skin is abolished, is due to the net transport of sodium ions from outside to inside of the skin.

Since the existence of two types of adrenoceptors, α & β, in effector tissues, such as smooth muscle, heart muscle and salivary glands, was proposed by Ahlquist (1948), several investigators have tried to confirm the existence and the involvement of adrenoceptors in sodium transport in the frog skin. Koefoed-Johnsen et al.(1952) demonstrated in isolated skin that epinephrine produced increases in SCC & sodium influx, however Na outflux also increased. Watlington et al. (1965, 1968, 1971) observed the adrenergic influence of adrenaline and isoproterenol on Na & Cl transport and concluded that both α- and β-adrenoceptors are involved in Na transport in frog skin. Pinschmidt et al. (1973) also reported that norepinephrine augmented transports of both Na & Cl in the frog skin, however they claimed that β-adrenoceptor are responsible for the stimulation, on the ground that propranolol, a β-adrenergic antagonist, blocked the stimulatory effects of NE. However, the assumption on which their conclusion have been founded is contradictory and flawed in view of the present knowledge of adrenergic system, a more specific agonists and antagonists, with which the types of adrenoceptors can be distinctly differentiated, have been introduced in the mean time. It was therefore attempted in the present study to substantiate the existence of distinctive types of adrenoceptors and to delineate their physiological roles in sodium transport of the frog skin with aid of more specific agonists and antagonists.

MATERIALS AND METHOD

Rana nigromaculata of either sex weighing 30-50 g were used, and the experiments were carried out during warm seasons from April to October. After pithing the spinal cord, abdominal skin was resected out and the skin was placed between two identical lucite chambers with 3.14 cm² cross-sectional area, as described by Kim (1968). Both sides of the chambers were filled with 20 ml of Frog Ringer's solution and the solution was aerated with O_2.

In some experiments, it took many hours for PD & SCC to return to the control level after washing out the agents added. Therefore, in experiments with antagonist pretreatment the skin was halved vertically along the midline. Both halves of skin were placed separately and to one half-skin only agonist was added, whereas the other received antagonist before agonist. If the PD values differed more than 5 mV between both hemi-skins during the control periods, the preparations were discarded. All the experiments were carried out under room temperature of 21-25°C. For measurement of potential difference (PD) and short-circuit current (SCC), calomel half-cells were connected by KCl-agar bridges to the skin chambers by Ussing and Zerahn's technique (1951). Total skin conductance (TSC) was calculated by dividing SCC with open-circuit PD. After about 2 hrs of stabilization period, the agents were added to the fluid bathing the serosal side of the skin. After the drug administration, skin PD & SCC were measured at 5 or 10 min intervals with volt meter (Narco physiograph MK-IV) and micro-ammeter respectively. All the data were presented as fractional change from the control values. Frog Ringer solution contains 103 mM NaCl, 10 mM KCl, 0.9 mM CaCl₂, 1.2 mM NaHCO₃ and 5.5 mM glucose. Drug used are epinephrine bitartrate (Sigma), norepinephrine bitartrate (Sigma), phenylephrine hydrochloride (Sigma), isoproterenol hydrochloride (Sigma), clonidine hydrochloride (Sigma), guanabenz

acetate (Wyeth Lab Inc.), prazosin hydrochloride (Pfizer), phenoxybenzamine hydrochloride (Smith, Kline & French Co.), dl-propranolol hydrochloride (Sigma) and yohimbine hydrochloride (Merck). Statistical significance was tested with Student's paired *t-test* for the changes from the control period, and when comparing two groups of experiments unpaired *t-test* was employed (Snedecor & Cochran, 1980).

RESULTS

1. Effects of various adrenergic agonists on PD, SCC and TSC of frog skin

Within several seconds after the administration of 6×10^{-6} M norepinephrine, both the PD & SCC started to increase, nearly doubling during 5-10 min period from the control values of 22 mV and 48.5 μA, resp. Then they returned gradually to the initial levels in 50 min. Repeated administration of NE after 2 hours elicited responses similar to the first one (Fig. 1).

The responses of PD & SCC to NE increased with increasing doses, as summarized in Table 1. The maximal response was observed with a concentration of 6×10^{-6}M. TSC was little affected, as both PD & SCC increased proportionately. The response to phenylephrine (PE), a specific α_1-adrenoceptor agonist, was summarized in Table 1. Also here, PD & SCC increased in a dose-dependent fashion but much higher concentrations were required to elicit roughly equal responses as with NE. And the increase lasted longer than with NE. Epinephrine (Epi), an agonist of both α- & β-adrenoceptor, produced increase of PD & SCC in a dose-related fashion similar to the cases with NE and PE, but the PD increased more markedly than SCC, and TSC tended to decrease (Table 2).

Table 3 is the summary of the data of isoproterenol, a specific β- adrenoceptor agonist, on PD & SCC of the frog skin. With concentrations ranging from 5×10^{-7}M to 5×10^{-6}M isoproterenol produced dose-related decrease in PD & SCC, which recovered to control values in about 50 min. There was no significant change in TSC.

2. Influence of α-adrenoceptor blockade on the effects of α-agonists

To ascertain the involvement of adrenergic receptors in the action of NE, PE and Epi, the influence of prazzosin, a specific α_1-adrenoceptor blocker, upon the effect of the above agents were investigated. Prozosin 2×10^{-6}M alone did not elicit any appreciable changes of PD & SCC. Fig. 2 depicts the effects of NE, PE and Epi on PD & SCC of prazosin-pretreated skin as compared with those of non-treated
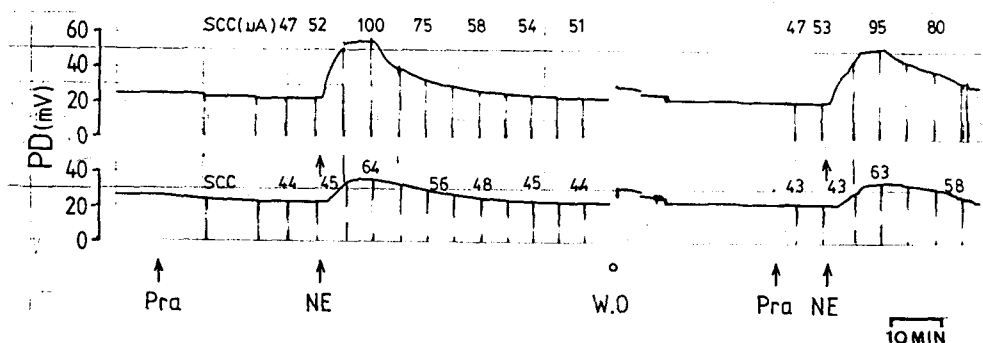

Fig. 1. An experiment showing the effects of norepinephrine(NE) on potential difference (PD, mV) and short-circuit current (SCC, μA) of the frog skin, and the influence of prazosin pretreatment on the effects. Upper tracing represents the control side of the paired hemiskin preparation treated with NE 6×10^{-7}M. Lower tracing represents the experimental hemiskin pretreated with prazosin(Pra) 2×10^{-6}M, 30 min before NE administration. At arrow, the agents indicated are added to the fluid bathing in serosal side. At o, chambers are washed out three times with fresh frog Ringer's solution during 60 min.

Table 1. Effects of norepinephrine(NE) and phenylephrine(PE) on potential difference(PD) and short-circuit current(SCC) of the frog skin

Drugs	Dose(M)	n	PD Control (mV)	PD 5 min Δ%	PD 10 min Δ%	PD 20 min Δ%	PD 30 min Δ%	SCC Control (µA)	SCC 5 min Δ%	SCC 10 min Δ%	SCC 20 min Δ%	SCC 30 min Δ%
NE	6×10^{-8}	8	26.1	+10.2ᵇ	+12.4ᵇ	+11.7	+12.4ᵇ	58.6	+14.2ᵃ	+14.1ᵇ	+8.9ᵇ	+7.2ᵃ
			±3.0	±2.8	±3.1	±3.5	±3.8	±6.2	±4.3	±3.8	±2.0	±2.4
	6×10^{-7}	10	23.8	+43.6ᵇ	+58.9ᶜ	+44.2ᶜ	+29.4ᶜ	51.7	+54.0ᶜ	+64.7ᶜ	+44.6ᶜ	+24.6ᵇ
			±2.3	±9.2	±11.5	±7.1	±5.6	±5.0	±9.7	±11.6	±8.5	±6.7
	6×10^{-6}	8	21.4	+106.1ᵇ	+140.7ᶜ	+103.9ᵇ	+72.4ᶜ	43.8	+109.8ᶜ	+142.1ᶜ	+112.5ᶜ	+81.8ᶜ
			±2.6	±22.2	±25.8	±19.3	±13.2	±4.1	±18.0	±18.3	±15.1	±9.8
	6×10^{-5}	6	27.3	+82.6ᵃ	+113.2ᵇ	+106.4ᵃ	+99.7ᵃ	50.8	+102.3ᵇ	+113.5ᵇ	+108.7ᵇ	+98.8ᵃ
			±5.4	±24.7	±27.2	±30.7	±29.1	±10.2	±23.0	±24.1	±24.4	±22.7
PE	5×10^{-6}	5	11.2	+18.6	+36.7ᵃ	+32.5	+33.4	23.5	+28.5	+31.5ᵇ	+30.4	+25.2
			±2.0	±8.0	±11.6	±8.9	±13.4	±3.4	±15.2	±6.8	±11.6	±9.1
	5×10^{-5}	6	12.8	+34.1ᵇ	+50.3ᵇ	+65.3ᵇ	+54.9ᵇ	25.8	+29.4ᶜ	+47.1ᵇ	+57.7ᵇ	+46.4ᵇ
			±2.8	±7.1	±8.1	±10.6	±8.4	±4.5	±3.7	±8.0	±10.0	±7.0
	1.5×10^{-4}	5	16.2	+76.3	+113.6	+120.4	+115.9	27.9	+53.2ᵃ	+101.1ᵃ	+90.1	+93.5
			±6.4	±29.5	±52.6	±63.0	±69.5	±8.4	±15.2	±25.9	±39.3	±44.9
	5×10^{-4}	5	12.5	+103.2	+125.2	+97.4ᵃ	+74.4ᵃ	24.5	+89.7	+112.8	+95.6ᵃ	+68.0ᵃ
			±3.0	±63.8	±62.0	±28.7	±17.9	±5.1	±52.7	±55.5	±27.8	±18.2

Values represent the mean ± S.E. for the number (n) of experiments indicated. Δ%: percent change of values from the control. Significance tested with "t"-test for paired differences from control values. ᵃ = $p<0.05$, ᵇ = $p<0.01$ and ᶜ = $p<0.001$.

Table 2. Effects of epinephrine on PD, SCC and total skin conductance (TSC) of the frog skin

Dose(M)	n	PD Control (mV)	PD 5 min Δ%	PD 10 min Δ%	PD 20 min Δ%	SCC Control (µA)	SCC 5 min Δ%	SCC 10 min Δ%	SCC 20 min Δ%	TSC Control (mmho)	TSC 5 min Δ%	TSC 10 min Δ%	TSC 20 min Δ%
5.5×10^{-7}	8	22.4	+15.5	+17.4	+12.9ᵃ	44.3	+12.6	+12.6	+10.4	2.01	−1.6	+3.3	−1.0
		±3.0	±7.3	±7.1	±4.6	±5.1	±9.5	±8.6	±5.4	±0.13	±4.1	±4.0	±3.5
5.5×10^{-6}	11	21.3	+64.7ᵃ	+89.4ᵇ	+66.7ᵃ	43.2	+50.7ᵇ	+64.5ᵇ	+39.6ᵃ	2.07	−3.8	−5.5	−2.2
		±2.4	±21.9	±25.5	±23.1	±4.1	±11.4	±15.1	±13.0	±0.10	±7.0	±6.2	±6.8
5.5×10^{-5}	6	19.9	+105.2ᵃ	+123.1ᵃ	+103.4ᵃ	42.2	+72.0ᶜ	+75.6ᵃ	+57.6ᵃ	2.20	−11.6	−14.4	−14.9
		±3.5	±32.4	±40.0	±35.9	±6.3	±6.8	±20.6	±15.3	±0.15	±9.0	±9.4	±9.8

Legends are the same as in Table 1.

skin. The increments of PD & SCC were significantly inhibited by prazosin and the dose-response curve were shifted to the right. Under the influence of prazosin, Epi produced a slight transient decrements of PD & SCC immediately after the drug administration, which may be due to β-adrenergic stimulation. This point was fortified by the observation with phenoxybenzamine (PBZ), an irreversible α-adrenoceptor blocker, as shown in Fig. 2. The stimulatory effects of PD & SCC were completely abolished by PBZ. Furthermore, with larger doses of Epi the response was reversed to a decrease.

Table 3. Effects of isoproterenol on PD and SCC of the frog skin

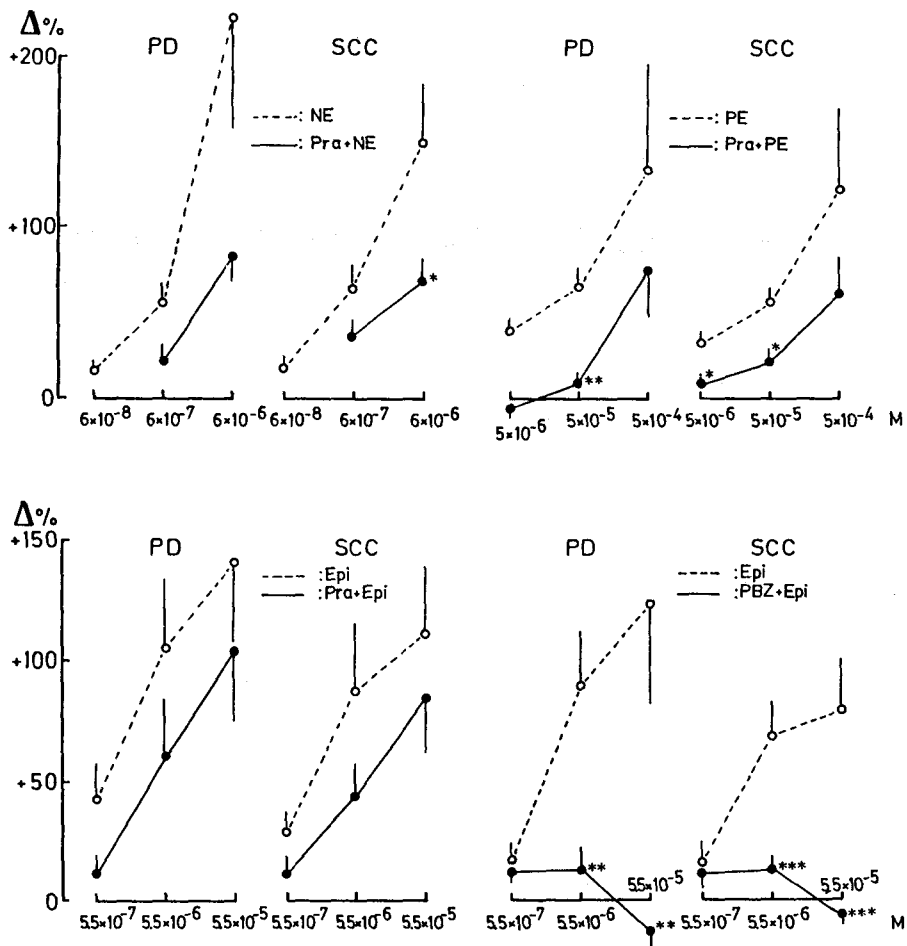| Dose(M)	n	Control (mV)	5 min	10 min	20 min	30 min	Control (μA)	5 min	10 min	20 min	30 min
		PD		Δ%			SCC		Δ%		
5×10^{-7}	5	30.9 ±6.1	−9.2 ±3.5	−7.1 ±3.5	−7.6[a] ±2.3	−7.8[b] ±1.5	61.2 ±14.0	−2.9 ±4.0	−0.9 ±2.0	−6.8 ±5.2	−7.7 ±6.1
1.5×10^{-6}	5	26.7 ±6.0	−17.9 ±6.9	−15.3 ±6.5	−14.7 ±5.8	−13.5 ±5.1	44.2 ±4.2	−18.1 ±9.1	−4.1 ±7.4	−4.6 ±9.0	−2.5 ±6.6
5×10^{-6}	5	30.2 ±6.8	−24.9 −11.7	−12.4[a] ±4.2	−12.0[a] ±4.0	−10.4 ±5.1	59.2 ±14.7	−25.1 ±13.2	−4.5 ±7.9	−6.6 ±9.2	−0.7 ±13.5

Legends are the same as in Table 1.

Fig. 2. Influence of prazosin (Pra, 2.6×10^{-6}M) and phenoxybenzamine (PBZ, 3.3×10^{-5}) upon the effects of NE, PE and Epi on PD & SCC of frog skin. Asterisks indicate significant difference between corresponding values of both groups (*= $p<0.05$, *= $p<0.01$ and *= $p<0.001$). Other legends are the same as in the previous figures.
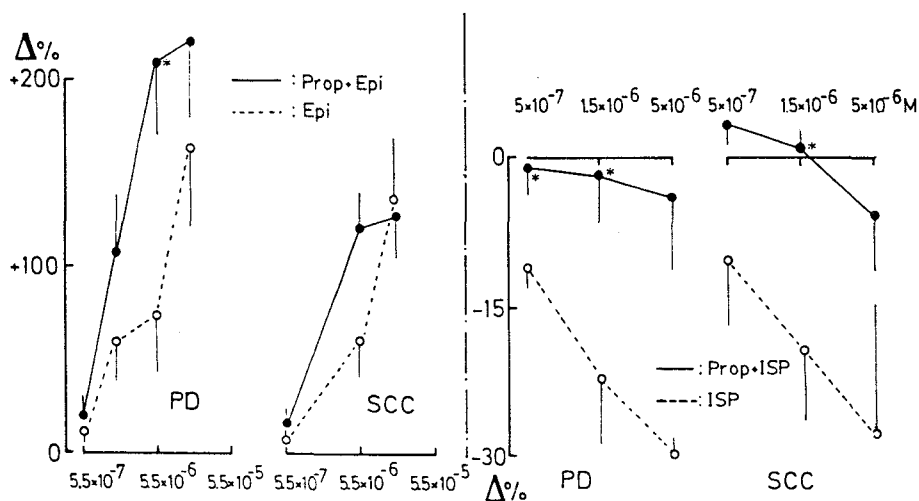
Fig. 3. Influence of propranolol (Prop, 4×10^{-6}M) on the effects of epinephrine(Epi) and isoproterenol(ISP) on PD and SCC. Other legends are the same as in the previous figures.

3. Influence of propranolol upon the effects of Epi and ISP

Fig. 3 illustrates the influence of propranolol, a specific β-adrenoceptor blocker, on the effects of Epi and isoproterenol (ISP) on PD & SCC. As shown here, the increments of PD & SCC by Epi were markedly potentiated and the effects of ISP on PD & SCC were completely abolished by propranolol pretreatment. The concentration of propranolol used here, 4×10^{-6}M, did not show any changes in PD and SCC.

4. Effects of clonidine and guanabenz on PD and SCC

Both clonidine and guanabenz, α_2-adrenoceptor agonists, produced increases in PD & SCC but did not show the dose-response relationship. And TSC was little affected (Table 4).

Table 4. Effects of clonidine(Clo) and guanabenz(Gua) on PD and SCC of the frog skin

| Drugs	Dose(M)	n	PD					SCC				
			Control (mV)	5 min	10 min	20 min	30 min	Control	5 min	10 min	20 min	30 min
				$\triangle\%$					$\triangle\%$			
Clo	4.3×10^{-6}	7	19.9	± 2.2	-1.2	-7.5	-8.2	45.1	$+0.1$	-3.1	-13.1	-13.5
			± 2.4	± 3.1	± 5.0	± 7.0	± 7.4	± 4.5	± 2.5	$\pm 5.7^b$	± 6.2	± 7.8
	4.3×10^{-5}	8	15.8	$+40.4^a$	$+61.5^a$	$+39.9^a$	$+22.4$	33.0	$+27.7^a$	$+37.5^b$	$+21.8^a$	$+8.1$
			± 3.2	± 14.1	± 19.6	± 12.4	± 11.3	± 7.1	± 11.4	± 10.6	± 8.9	± 7.8
	4.3×10^{-4}	9	23.1	$+49.2^a$	$+73.1^a$	$+61.4$	$+41.4$	47.8	$+27.4^b$	$+35.8^b$	$+25.5$	$+13.5$
			± 3.3	± 5.7	± 24.8	± 30.5	± 24.4	± 6.2	± 6.6	± 9.9	± 11.6	± 9.8
Gua	4.3×10^{-5}	10	20.1	$+12.4$	$+42.6^b$	$+50.3$	$+24.8$	41.5	$+10.6^a$	$+34.0^b$	$+34.6^a$	$+11.7$
			± 2.1	± 6.9	± 11.1	± 23.4	± 24.2	± 3.2	± 3.7	± 8.4	± 13.0	± 14.2

Legends are the same as in Table 1.
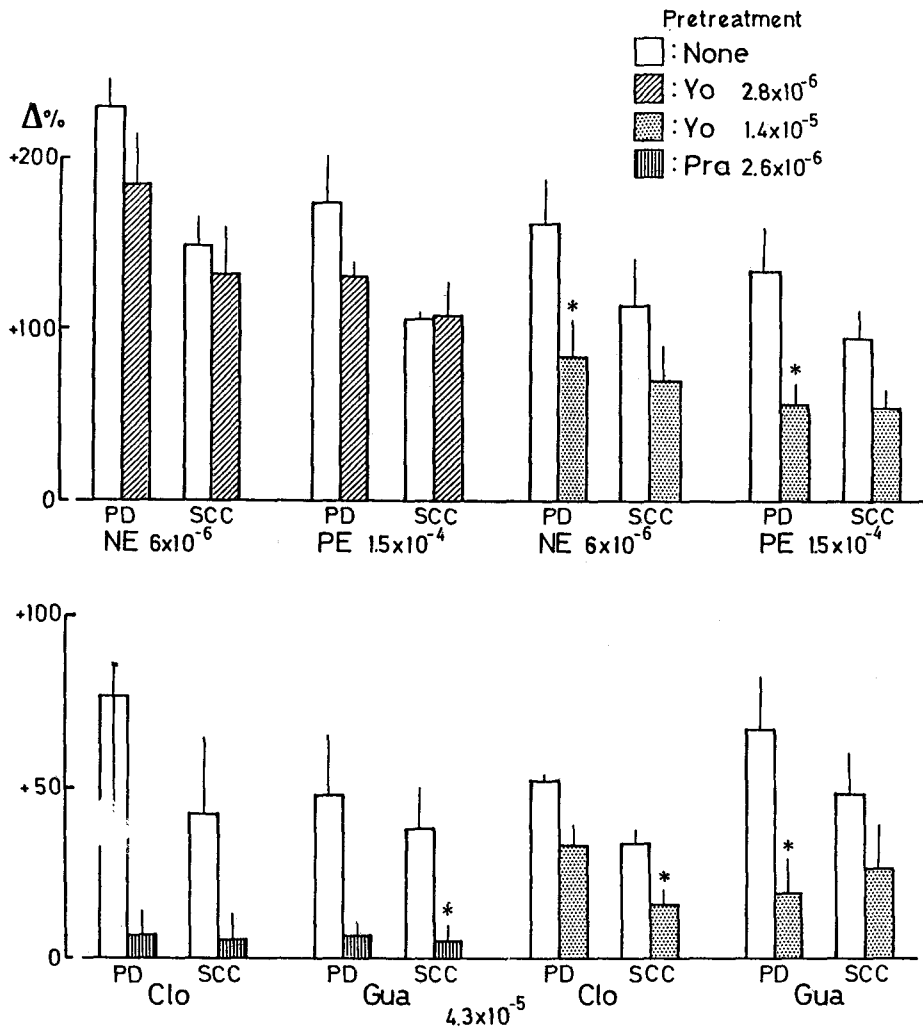
Fig. 4. Influence of prazosin(Pra) and yohimbine(Yo) upon the effects of norepinephrine(NE), phenylephrine(PE), clonidine(Clo) and guanabenz(Gua) on PD & SCC. Asterisks (≒ p<0.05) indicate significant difference between corresponding values of both groups. Other legends are the same as in the previous figures.

5. Influence of prazosin and yohimbine upon the effects of NE, PE, clonidine and guanabenz

Yohimbine, in concentration of 2.8×10^{-6}M or 1.4×10^{-5}M did not show any changes of PD & SCC. PD & SCC increments by NE and PE were not influenced by low concentration (2.8×10^{-6}M) of yohimbine but were inhibited by high concentration (1.4×10^{-5}M). The effects of clonidine and guanabenz on PD & SCC were inhibited by high concentration of yohimbine. Prazosin completely abolished the effects of clonidine and guanabenz (Fig. 4).

DISCUSSION

Since it was known that the frog skin represents a prototype of epithelial systems which transport ions from one border of the cell to the other (Huf, 1935; Krogh, 1937, 1938), a large body of experimental

data on the patterns and mechanism of this process has accumulated (Koefoed-Johnsen *et al.* 1953; Kirschner, 1955; Engbaek *et al.,* 1957). Based on various lines of evidence, Koefoed-Johnsen and Ussing (1958) proposed a model of the frog skin. According to it, the outer (epidermal) membrane of the epithelial cell of stratum germinativum is selectively permeable to Na^+ and to Cl^- (to a lesser degree) but impermeable to K^+, whereas the inner membrane is permeable to K^+ and impermeable to Na^+. The sodium pump located at the inner membrane pumps out Na^+ in exchange for K^+ (hence "non-electrogenic"), and K^+ pumped in leaks out of the inner membrane. Thus the PD difference across the whole skin is equal to the sum of Na^+-diffusion potential at the outer membrane and K^+-diffusion potential at the inner membrane which depends on the Na^+-pump activity. The SCC is concordant with net Na^+ transport across the skin and is dependent on the sodium pump activity. However, because the pump is not always saturated with Na^+, the permeability change of external membrane could also affect the SCC (Koefoed-Johnsen *et al.,* 1953; Kirschner, 1955; Ussing, 1965). It is thus assumed that PD increment may result either from decreased permeability of the external membrane or from increase in the sodium pump activity. PD decrement will follow if increased permeability of the external membrane enhance Na-pool in the cell, or if the pump is depressed. The increment of SCC, however, will result either from increased permeability or from increased pump activity, while decreased SCC will entail if the permeability is decreased or the pump activity is diminished.

Our observation show that α-adrenergic agonists, NE and PE, produced marked elevations of PD & SCC without significant changes of TSC, which is best explained if one assumes that they stimulated the pump activity rather than changes in permeability of the inner membrane. These stimulatory effects of NE and PE were inhibited competitively by prazosin, a specific α_1-adrenoceptor blocking agent, indicating that α-adrenoceptors are involved in the stimulatory action of the Na pump. On the other hand, isoproterenol, a specific β-adrenoceptor agonist, produced dose-dependent decreases in PD & SCC with no changes in TSC, and these effects are abolished by propranolol-pretreatment, indicating that β-adrenoceptors mediate the decreased activity of the sodium pump. Our data on Epi, which is known to stimulate both α- and β-adrenoceptors, can also be explained by the assumption that α-adrenoceptor mediate the stimulation whereas β-adrenoceptor is involved in effecting depression of the pump. Epi is less potent than NE, though it also elicits increases in PD & SCC, suggesting predominance of alpha effects. Also, in accordance with the premise is the fact that α-adrenergic blockade not only abolished the stimulatory action of Epi on PD & SCC but also revealed the inhibitory effects with higher doses. Furthermore, propranolol augmented increases in PD & SCC.

There are, however, several points not to be overlooked in the action of Epi. The increases in PD is greater than that of SCC, thus TSC tended to decrease after Epi. Also, PD increase was more markedly potentiated after propranolol pretreatment than SCC increase. Furthermore, increases of PD & SCC were observed with higher concentrations of isoproterenol (unpublished observation). These facts are difficult to account for by the model proposed by Koefoed-Johnsen and Ussing (1958). One of the possible mechanisms may be that epinephrine also affects other mechanism such as chloride outflux, as shown by Koefoed-Johnsen *et al.* (1953) and Watlington *et al.* (1965, 1968, 1971).

There have been several reports on the adrenergic influence upon the ion transport of frog skin. Ussing and Zerahn (1951) observed increases in sodium outflux as well as influx upon adding adrenaline. Koefoed-Johnsen *et al.* (1953) demonstrated that there exists a non-sodium current contributing to SCC and PD across the frog skin, and Epi increases this non-sodium current markedly, in addition to the increases in both influx and outflux of sodium. They also showed that the non-sodium current can be mostly ascribed to chloride transport in the outward direction and postulated that mucous gland transports chloride actively. Watlington *et al.* (1965, 1968, 1971) observed the effects of adrenaline and isoproterenol on the ion transport of frog skin, and postulated that α-receptors are inhibitory, whereas β-receptors are stimulatory on the ion transport. Pinschmidt *et al.* (1973) also reported that norepinephrine increased Na^+ and Cl^- transport but claimed that β-adrenoceptors mediate the stimulatory action while stimulation of α-adrenoceptors produce inhibition based on the observation that propranolol abolished the stimulatory

effects of norepinephrine. These reports are not consistent with our data which clearly indicate that α-receptors are stimulatory whereas β-receptors are inhibitory to PD & SCC. The discrepancy may have been caused by the fact that they tested only one dose of isoproterenol which produced stimulation, apparently unaware of dose-related reversal of the action. Also questionable is their interpretation of specificity of agonists and antagonists to adrenoceptors, and reevaluating their data in light of present knowledge as well applying new, more specific antagonists would lead them to a different conclusion. The differences in the methods and species used (they used Rana pipiens) may also have contributed in the discrepancy.

Alpha receptors are shown to consist of two kinds of subtypes, α_1 and α_2, the former being more sensitive to phenylephrine and the latter to clonidine (Berthelson et al., 1977; Starke et al., 1977; Wikberg, 1978). Therefore, we tried to characterize the subtype of α-adrenoceptor participated in the Na^+ transport of the frog skin, by observing the effects on PD & SCC of clonidine and guanabenz, specific α_2-adrenoceptor agonists, and also the influence of prazosin and yohimbine, α_1- and α_2-adrenoceptor blocker, respectively, on their effects as well as on the effects of NE & PE. The PD & SCC increments of NE and PE were not influenced by low concentration of yohimbine, but they were inhibited by higher doses. High concentration of yohimbine were needed to inhibit the effects of clonidine and guanabenz on PD & SCC, while only lower concentration of prazosin was required to abolish the effects of clonidine and guanabenz. These observations can be easily accounted for, if one assumes that clonidine and guanabenz also act on α_1-adrenoceptor and higher doses of yohimbine could block α_1-adrenoceptor also. Or, α-adrenoceptors on the frog skin may not have further differentiated into α_1- and α_2-sybtypes, as in the higher animals. In this case, prazosin should block the adrenoceptor more efficiently than yohimbine.

Overall, our present observations suggest that there exists in the frog skin two distinctive types of adrenoceptors, and that α-adrenoceptors mediate the stimulatory effects of PD & SCC, whereas β-adrenoceptors are responsible for the inhibitory effects. However, no evidence was provided which would suggest further differentiation of alpha adrenoceptors into subtypes.

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= 국문초록 =

개구리 피부에 있어서 Na 수송을 조절하는 Adrenoceptors에 관한 연구

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본 연구에서는 개구리(Rana nigromaculata)의 피부에 있어서 전위차(PD), 단락전류(SCC) 및 total skin conductance(TSC)에 미치는 제종 adrenergic agonist 및 그 차단제의 영향을 관찰하여 개구리 피부에 adrenoceptors의 존재를 확인하고 Na 수송에 있어 그들의 역할을 구명코자 하였다.

1. Norepinephrine(NE, $6 \times 10^{-8} - 6 \times 10^{-5}$M), phenylephrine(PE, $5 \times 10^{-6} - 5 \times 10^{-4}$M)의 PD 및 epinephrine(Epi, $5.5 \times 10^{-7} - 5.5 \times 10^{-5}$M)의 PD 및 SCC 증가효과는 약물의 투여농도에 비례하였으며, Epi의 최대효과는 NE나 PE의 것보다 약하였다.

2. 이러한 PD 및 SCC의 증가효과는 alpha 1 adrenoceptor 차단제인 prazosin 2×10^{-6}M에 의해서 억제되었으며, 특히 Epi의 증가효과는 불가역성 alpha receptor 차단제인 phenoxybenzamine 3.3×10^{-5}M에 의하여 완전히 차단되며 대량의 Epi에 의해서는 PD 및 SCC의 감소를 초래하였다.

3. Beta adrenoceptor agonist인 isoproterenol($5 \times 10^{-7} - 5 \times 10^{-6}$M)에 의해 농도증가에 비례한 PD 및 SCC의 감소가 일어났으며, 이는 선택적 beta receptor 차단제인 propranolol 4×10^{-6}M에 의해 차단되었다. 또한 Epi의 PD 및 SCC 증가효과는 propranolol 4×10M에 의하여 강화됨을 볼 수 있었다.

4. Alpha 2 adrenoceptor agonist인 clonidine 및 guanabenz도 PD 및 SCC의 증가를 가져왔으며 이러한 효과는 alpha 2 receptor 차단제인 yohimbine에서 보다 Alpha 1 receptor 차단제인 prazosin에 의해 더 잘 억제되었다.

이상 실험의 결과 개구리 복부피부에도 포유동물에서와 같이 adrenergic alpha 및 beta receptor가 존재하며 alpha receptor는 PD 및 SCC의 증가를, beta receptor는 PD 및 SCC의 감소를 매개하여, 개구리 피부의 Na 수송에 있어 adrenergic system이 중요한 조절작용을 하고 있음을 알 수 있었다. 그러나 여기에 관여하는 alpha adrenoceptor는 다른 포유류에서와 같이 alpha 1 및 alpha 2 adrenoceptor로 구분할 수는 없었다.