BBA 75399

THE ACTION OF BETA ADRENERGIC SITE STIMULATING CATECHOLAMINES ON ISOLATED FROG SKIN

ROBERT D. McAFEE

VA Hospital and Physiology Department, Tulane University School of Medicine, New Orleans, La. (U.S.A.)

(Received August 25th, 1969)

SUMMARY

Living frogs injected with the beta adrenergic site stimulating catecholamine ethylnorepinephrine secrete a mucous containing equal amounts of Na^+ and Cl^-. Isolated short circuited frog skin treated with beta adrenergic site stimulating catecholamine, including protokylol (Caytine) and isopropylarterenol, produce an outflux of Na^+ equal to a concomitant outflux of Cl^- as measured with radioisotopes of Na^+ and Cl^-. Na^+ influx and short circuit current is also stimulated equally. We conclude that two independent mechanisms are stimulated by these catecholamines: when used at 0.1 μM concentrations, Na^+ transport is stimulated; when used at 10 μM concentrations, a secretion of mucous containing Na^+ and Cl^- is additionally stimulated. This conclusion is not in agreement with the results of a previously published investigation. In that study isopropylarterenol was reported to stimulate the production of a non-Na^+ short circuit current, presumably of Cl^-. The conflict results, perhaps from not using radioisotopic Cl^- in the investigation and therefore missing correspondence between Na^+ and Cl^- outflux, and between Na^+ influx and short circuit current.

INTRODUCTION

It is of continuing interest to extend our knowledge of the effect of alpha and beta adrenergic site stimulating catecholamines on different tissues. Since isolated frog skin is a convenient tissue for the study of Na^+ transport it is important to determine if these catecholamines modify Na^+ transport in this tissue. The effect of epinephrine has been described as stimulating Cl^- outflux (Cl_0^-) from the inside of the skin outward to the pond side of the skin[1]. It has also been reported that the beta adrenergic site stimulating catecholamine isopropylarterenol stimulates Cl_0^- rather than Na^+ influx (Na_i^+) in isolated frog skin[2]. In the study with isopropylarterenol, Na_i^+ and Na^+ outflux (Na_0^+) were measured using radioisotopes of Na^+. It was found that isopropylarterenol increased both these fluxes. The isolated frog skin short circuit current (I_{sc}) was also increased. Although both I_{sc} and Na_i^+ increased nearly equally, the increased I_{sc} was not attributed to the increase in Na_i^+ because of a concomitant increase in Na_0^+. According to the net flux equation of USSING AND ZERAHN[3], $I_{sc} = Na_i^+ - Na_0^+$. Thus the short circuit current is the algebraic sum of the ion fluxes. Since both Na_i^+ and Na_0^+ increased about equally in the isopropylarterenol experiments, it was thought that the increased I_{sc} observed was not the result of Na_i^+ but of stimulation of the flux of some other ion, probably Cl_0^- as previously found for epinephrine. The increased Na_i^+ and Na_0^+ was believed to be the result of an increased permeability of the skin to passive diffusion of these ions. Cl^- outfluxes were not measured in these experiments, however. We disagree with these conclusions and have repeated the isopropylarterenol experiments using radioisotopes of Cl^- as well as of Na^+ in order to determine if Cl_0^- is responsible for the stimulated increase in short circuit current. Other beta adrenergic site stimulating catecholamines were also investigated to determine if the effect we found, stimulation of I_{sc} and Na_i^+ with an independently elicited stimulation of mucus containing Na^+ and Cl^-, was the characteristic action of these catecholamines on frog skin.

MATERIALS AND METHODS

Rana pipiens from a northern supplier were stored in stainless steel pans with a slow trickle of deionized water for 2–3 weeks. After sacrifice the abdominal skin of each frog was divided into a right and left half and placed as a membrane separating two conical lucite chambers in an apparatus similar to that described by USSING AND ZERAHN[3]. Ringer's solution (25 ml) bathing both sides of the skin was circulated and aerated by an air lift pump. Each l of Ringer's solution used in these experiments contained NaCl, 109.4 mM; $NaHCO_3$, 2.8 mM; KCl, 1.88 mM; $CaCl_2$, 1.08 mM and $MgCl_2 \cdot 6\ H_2O$, 0.5 mM. In some experiments deionized water (from a Continental Water Service Deionizer yielding a value of less than 0.1 ppm as NaCl by Barnstead Purity Meter determinations) was used to fill the chamber bathing the outside of the frog skin.

A short circuiting device similar to that described by USSING AND ZERAHN[3] was used in these experiments. A Keithley d.c. VTVM model 200B (Keithley Instruments, Cleveland, Ohio) was used to read the potential difference across the frog skin, and the skins were continuously short circuited except when potential difference measurements were being taken or when deionized water was used. The measured short circuited current was corrected for the effect of the resistance of the bathing medium in the following manner: (1) Total resistance = measured potential difference/measured I_{sc}. (2) Skin resistance = total resistance − solution resistance between potential difference electrodes. (3) Corrected I_{sc} = measured potential difference/skin resistance.

The short circuit is then adjusted to the corrected I_{sc} and left on this setting until the next reading is taken.

Varying concentrations of catecholamines were used in order to obtain the effect desired. Isopropylarterenol (City Chemical Corporation) was used at 10 and 0.1 μM to demonstrate the effect of concentration. Caytine (Lakeside Laboratories), ethylnorepinephrine (Breon Laboratories, Bronkephrine·HCl), and epinephrine (Parke Davis and Co., adrenalinchloride) were used at 0.1 μM. The drug was added to the solution bathing the inside of the frog skin.

Mucous was collected by immobilizing the frogs on a dissection board. Following a period of accommodation, epinephrine or ethylnorepinephrine was injected under the skin at a concentration of 0.5 mg in 0.5 ml 0.8 % NaCl solution. Mucous was removed from the abdominal skin with a spatula. Samples were taken every 30 min. Analysis of Na^+ and Cl^- from mucous secretion from live frogs was done using a Coleman flame photometer and a Buchler Cotlove chloridometer.

$^{22}Na^+$ (Abbott Laboratories) and $^{36}Cl^-$ (Nuclear Research Chemicals, Inc.) were used in experiments because of their advantageous long half-lives. In some experiments influx measurements were made with both isotopes simultaneously on one-half of the abdominal skin and simultaneous outflux measurements were made on the other half skin. The isotope tracer procedures are similar to those described by others[2,3,5,6]. 100 µl of $^{36}Cl^-$ with total activity of 2.5 µC or 500 µl of $^{22}Na^+$ with total activity of 10 µC were added to the bathing medium except in the experiments with deionized water outside, when 1/10 of these doses were used. 500 µl were removed at 30-min intervals. The $^{36}Cl^-$ samples were added to 15 ml of scintillation (2,5-bis-2-(5-*tert.*-butylbenzoxazolyl)thiophene) solution and counted and recorded with a Packard Instrument Co. automatic scintillation detector. The $^{22}Na^+$ samples were counted and recorded with a Tracerlab gamma spectrometer and automatic well sample changer. The counts contributed by each isotope were separated by counting both together in the scintillation detector and then in the gamma spectrometer which counted only the $^{22}Na^+$. Cl^- and Na^+ fluxes were converted into µA/cm² (factor: 1 µequiv = 26.8 µA) for comparison with the I_{sc} data.

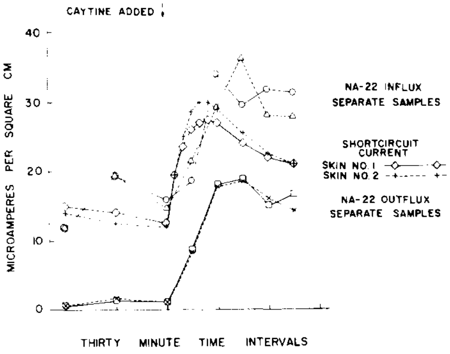
The frog skins were allowed to stabilize for 2 h before isotope was added and 30 additional minutes were allowed before taking the first sample. Three isotope flux values were taken before adding the catecholamine to the inside bathing medium. I_{sc} was continuously monitored so that a drift in potential difference over 2 mV did not occur. Two samples of radioisotope bathing medium were taken at each period to indicate the degree of precision involved in these experiments.

Tables I and II were constructed from measurements taken from experiments similar to those shown graphically in the figures. The values entered in the tables were those observed immediately before adding catecholamines and those taken 30 min later. As is evident from the graphs, such values are fair representations of the events being observed.

RESULTS

Mucous was collected from frogs 1 h after ethylnorepinephrine was injected subdermally. It was found that the frogs secreted a mucous which contained both Na^+ and Cl^- in approximately equal concentrations: In five experiments, $Na^+ = 36 \pm 4$ S.D. mequiv/l, $Cl^- = 33 \pm 8$ S.D. mequiv/l. These results show that there is an outflow *in vivo* of Na^+ and Cl^- due to ethylnorepinephrine stimulation. Some frogs were injected with adrenalin for comparison. These frogs showed an initial 30-min secretion of mucus which contained greater concentration of Cl^- than Na^+, as follows: In eleven experiments, $Na^+ = 19 \pm 4$ S.D. mequiv/l, $Cl^- = 52 \pm 17$ S.D. mequiv/l. In five additional 1-h experiments with epinephrine, the ratio had changed: $Na^+ = 69 \pm 25$ S.D. mequiv/l, $Cl^- = 38 \pm 14$ S.D. mequiv/l. These hourly findings with epinephrine confirm recently reported studies on frog skin secretions[4], as well as those originally reported by KOEFED-JOHNSEN *et al.*[1].

Figs. 1 and 2 show the effect of beta adrenergic site stimulation (Caytine) on the I_{sc} and Na^+ and Cl^- fluxes of isolated frog skin measured radioisotopically.



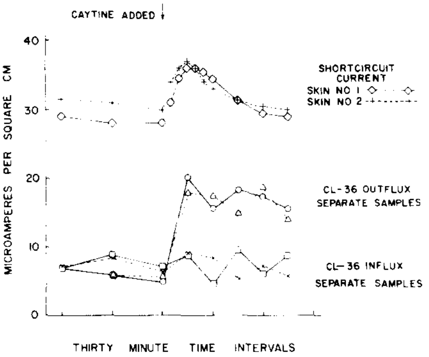


Fig. 1. Na^+ outflux and I_{sc} from one skin-half and Na^+ influx and I_{sc} from the other skin-half of a single frog. Skins bathed in Ringer's medium. Caytine added at arrow.

Fig. 2. Cl^- outflux and I_{sc} from one skin-half and Cl^- influx and I_{sc} from the other skin-half from a single frog. Skin bathed in Ringer's medium. Caytine added at arrow.

TABLE I

BETA ADRENERGIC SITE STIMULATING CATECHOLAMINE STIMULATION OF I_{sc} AND $^{22}Na^+$ AND $^{36}Cl^-$ FLUXES IN ISOLATED FROG SKIN STIMULATED WITH 10 μM ISOPROPYLARTERENOL

Values in $\mu A/cm^2$ ± S.D. prior to treatment (Pre Rx) and the change observed 30 min later (Δ). Eight experiments.

Skin-halves	*Value measured*	*Means in μA/cm²*	
		Pre Rx ± S.D.	*Δ ± S.D.*
16 skin-halves	I_{sc}*	22.3 ± 5.5	13.8 ± 5.4
8 left	$^{22}Na_i^+$	25.9 ± 5.1	13.6 ± 5.0
8 right	$^{22}Na_0^+$	1.7 ± 1.2	12.8 ± 2.1
8 left	$^{36}Cl_i^-$	32.0 ± 11.4	1.3 ± 9.3
8 right	$^{36}Cl_0^-$	23.1 ± 8.7	13.0 ± 5.4

* The I_{sc} is the mean of 16 skins (8 paired skin-halves). 8 left skin-halves were used for the simultaneous $^{22}Na_i^+$ and $^{36}Cl_i^-$ experiments and 8 right skin-halves for the simultaneous $^{22}Na_0^+$ and $^{36}Cl_0^-$ experiments.

Identical results are obtained with isopropylarterenol as summarized in Table I. Using 10 μM isopropylarterenol, the correspondence between I_{sc} and Na_i^+ is easily seen both before and after treatment. The increase in Na_0^+ and Cl_0^+ are seen to be essentially identical following treatment. Cl_i^+ remains unchanged. Table II shows that when 0.1 μM isopropylarterenol is used to stimulate isolated frog skin the I_{sc} increases in all of the half skins to the extent to which we have come to expect from this drug (*viz.*, Table I). The Na_i^+ initial value and its increase parallels the initial value and the increase of the I_{sc} for the same skin-half. The other entries in this table show that neither Na_0^+ or Cl_0^+ is stimulated by 0.1 μM isopropylarterenol.

In order to determine if Na^+ and Cl^- outflux would continue to be stimulated by isopropylarterenol in the absence of Na_i^+ and I_{sc} we bathed the outside of the skin in deionized water. Unter these conditions no short circuit current or Na_i^+ could take place. Fig. 3 and Table III illustrate that Na^+ and Cl^- continue to be

TABLE II

STIMULATION OF I_{sc} AND Na_i^+ BY 0.1 μM ISOPROPYLARTERENOL IN THE ABSENCE OF AN EFFECT ON Na^+ AND Cl^- OUTFLUX

I_{sc}, $^{22}Na_i^+$, $^{22}Na_0^-$ and $^{36}Cl_0$ values in μA/cm² prior to treatment (Pre Rx) with 0.1 μM isopropylarterenol and the change observed 30 min later (Δ). 2 skin-halves from each frog (1 and 2).

Skin-half	I_{sc}		$^{22}Na_i^-$		$^{22}Na_0^-$	
	Pre Rx	Δ	*Pre Rx*	Δ	*Pre Rx*	Δ
1	15	25	20	21	—	—
2	15	24	—	—	3	4
1	25	23			2	1
2	30	13			2	1
1	15	11			4	0
2	13	14			4	0
			$^{36}Cl_0^-$			
			Pre Rx	Δ		
1	30	20	—	—	1	1
2	25	10	43	−7	—	—
1	15	10	—	—	4	1
2	17	8	16	1	—	—

TABLE III

THE EFFECT OF ISOPROPYLARTERENOL ON Na^+ AND Cl^- FLUXES WHEN DEIONIZED WATER IS USED AS THE OUTSIDE BATHING MEDIUM IN BOTH SKIN-HALVES

$^{22}Na_0^-$ and $^{36}Cl_0^-$ prior to treatment with isopropylarterenol (Pre Rx) and the change (Δ) 30 min later. Values in μA/cm².

*Expt.**	*Skin-half 1* $^{22}Na_0^+$		*Skin-half 2* $^{36}Cl_0^-$	
	Pre Rx	Δ	*Pre Rx*	Δ
1	1	34	—	—
2	1	24	—	—
3	2	19	7	20
4	2	23	2	25
5	—	—	3	18

* Measurements were made in each experiment on 2 separate skin-halves from 1 frog.

secreted in approximately the same quantity into the deionized water bathing the outside of the skin as they are in experiments with Ringer's medium bathing both sides of the skin. Data from Fig. 3 is not included in Table III.

DISCUSSION

We have observed that an injection into living frogs of the beta adrenergic site stimulating catecholamine ethylnorepinephrine stimulates the flow of mucous which contains equal amounts of Na^- and Cl^-. Similarly, in isolated frog skins, ethylnorepinephrine, isopropylarterenol and Caytine at 10 μM concentration stimulate

Na^+ and Cl^- outflux equally. In addition, these substances stimulate Na_i^+ to an extent equal to the stimulated increase in I_{sc}. In other experiments we noted that the dose of isopropylarterenol could be adjusted to 0.1 μM concentration so that Na_i^+ and I_{sc} were again stimulated equally but there was no significantly increased outflux of Na^+ and Cl^-. These experiments suggest that Na^+ and Cl^- outflux is stimulated independently of the stimulated increase in Na_i^+ and I_{sc}. Experiments with deionized water bathing the outside of isolated frog skin and Ringer's medium bathing the inside show that isopropylarterenol-stimulated increase in Na^+ outflux is independent of Na_i^+ since Na^+ outflux (and Cl^- outflux) was unaffected by deionized water bathing the outside of the isolated frog skin. This is further evidence that the stimulated increase in Na^+ outflux is the result of a secretion of Na^+ and Cl^- from frog mucous gland. These experiments are in agreement with the results obtained by others with norepinephrine[5]. These authors have reported that there is also a dose effect with this substance, the higher dose stimulating frog skin gland secretion.

It is of course possible to interpret our results on the basis of a more generalized flux equation. For example, one might wish to write the following expression to take into account the Cl^- flux: $I_{sc} = Na_i^+ - Na_0^+ + Cl_0^- - Cl_i^-$. We do not favor this interpretation, however. It is only necessary to realize that Na^+ and Cl^- outflux, if they are equal, do not contribute to the I_{sc}. Furthermore, this equation as well as the net flux equation of USSING AND ZERAHN[3] requires that the radioisotopes used in the experiment to determine the values of Na_i^+, Na_0^+, Cl_i^- and Cl_0^- actually "trace" these ions. If some amount of the "tracer" traces a secretion of Na^+ and Cl^-, perhaps as an unionized or partially ionized component of frog skin mucous, the data so obtained should not be entered in the net flux equation. The question may also be raised whether as a result of capture, decapitation and pithing the frog, mucous secretion may be produced which continues for a period of time from the isolated frog skin. Radioisotopically determined Na^+ outflux values, for substitution in the net flux equation, may be inflated by the Na^+ secreted in this manner.

It is seen that Na^+ influx and I_{sc} can be stimulated independently of Na^+ and Cl^- outflux; also, Na^+ and Cl^- outflux can be produced independently of Na^+ influx and I_{sc}. These observations support the view that two separate and indepen-

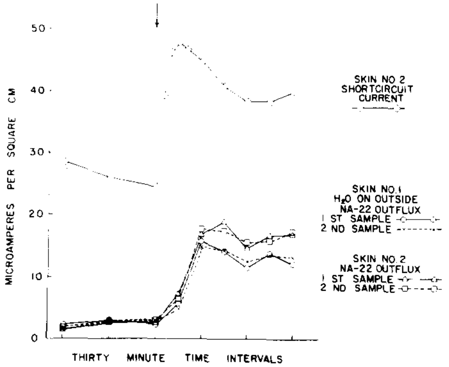


Fig. 3. Na^+ outflux and I_{sc} from one skin-half bathed with Ringer's medium on both sides, and Na^+ outflux from the other skin-half bathed in Ringer's medium on the inside and deionized water on the outside. Isopropylarterenol added at arrow.

dent mechanisms are stimulated by the action of beta adrenergic site stimulating catecholamines on isolated frog skin.

ACKNOWLEDGMENTS

The author wishes to thank Renee Bousquet and Lionel Cazenavette for their help in this experiment.

REFERENCES

1 J. Koefoed-Johnsen, H. H. Ussing and K. Zerahn, *Acta Physiol. Scand.*, 27 (1952) 38.
2 C. Watlington, *Am. J. Physiol.*, 214 (1968) 1001.
3 H. H. Ussing and K. Zerahn, *Acta Physiol. Scand.*, 23 (1951) 110.
4 R. T. Friedman, N. S. LaPorde, R. N. Aiyawar and E. G. Huf, *Am. J. Physiol.*, 212 (1967) 962.
5 F. Bastide and S. Jard, *Biochim. Biophys. Acta*, 150 (1968) 113.
6 C. F. Gonzalez, Y. E. Shamoo and H. R. Brodsky, *Am. J. Physiol.*, 213 (1967) 333.