Biophysical Observations

Synergistic Effect of Cyclopropane and Epinephrine on Sodium Transport in Toad Bladder

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In the short-circuited toad bladder cyclopropane enhanced active sodium transport whereas in bladders taken from toads pretreated with reserpine, cyclopropane was an inhibitor of sodium transport. Normal toad bladder contained a significant concentration of epinephrine which was reduced in animals pretreated with reserpine. Epinephrine alone stimulated sodium transport of the bladder and cyclopropane and epinephrine had a synergistic effect upon the ion transport. The stimulating effect of cyclopropane when used alone could be reversed by an α-blocker, but not by a β-blocker. When the concentration of α-blocker was high enough, cyclopropane inhibited sodium transport.

It has previously been shown for certain cellular membranes that local anesthetics,[1] halothane, and higher concentrations of diethyl ether inhibit, while lower concentrations of ether, cyclopropane, and nitrous oxide stimulate, the active transport of sodium.[2] The latter two gases gave rise to: (1) stimulation followed by (2) an added stimulation when the gases were discontinued, before (3) a slow return to baseline was seen (fig. 1). This was interpreted as representing simultaneous stimulation and inhibition, with the stimulation prevailing and the inhibition wearing off immediately upon discontinuation of the gases.

Ussing and Zerahn [3] showed that epinephrine is present in frog skin and stimulates sodium transport in this model. A synergistic effect of cyclopropane and epinephrine has repeatedly been shown.[4, 5] The purpose of this study therefore was to test whether or not the stimulating effect of cyclopropane on sodium transport is related to an interaction with catecholamines.

The toad bladder transports sodium from the mucosal to the serosal surface; it was chosen as a model in order to make possible a direct comparison between the results from this study and those reported earlier.[2]

Methods

The technique has been described in detail.[2] Bladders from toads (Bufo marinus) were divided. Each half was mounted between two symmetrical halves of a lucite chamber. The same bathing solution was used in both chambers: 110 m*M* NaCl, 10 m*M* KCl, 1 m*M* $MgCl_2$, 0.25 m*M* $CaCl_2$, 0.9 m*M* NaH_2PO_4, 4.3 m*M* Na_2HPO_4, 5.3 m*M* THAM, 2.2 m*M* HCl, 6 g./liter glucose, 0.75 g./liter adenosine, adjusted to *p*H 8.0 with 0.3 molar THAM. The *p*H did not change during an experiment.

Sodium transport gives rise to a transbladder potential, the mucosal side being electronegative. An external electromotive force (short-circuiting current or SCC), exactly nullifying the transmembrane potential, is directly related to active sodium transport.[2, 6] A Beckman expanded scale *p*H meter (membrane potential) and a d.c. microammeter (SCC) were used for continuous monitoring of the SCC. All experiments were carried out at ambient temperatures.

The following experiments were done:

1) Twenty-four toads were injected with reserpine, 10 mg./kg. intraperitoneally 90 and

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This paper was presented at the Work Completed Section of the Annual Meeting of the American Society of Anesthesiologists in Philadelphia, October 4, 1966. Accepted for publication October 12, 1966. This study was supported by Research Grant 5 R01 GM13029 and Career Development Award 1-K3-GM-33,049 from the National Institutes of Health.

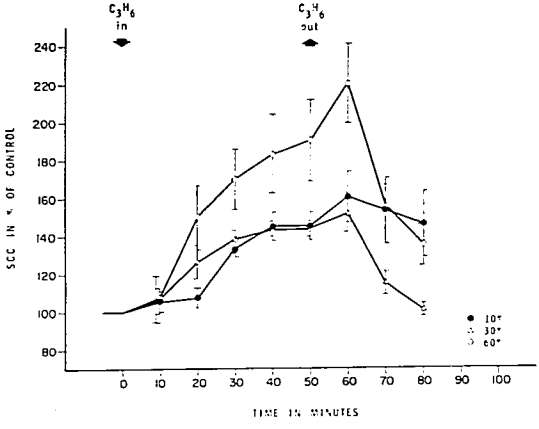

FIG. 1. The effect of cyclopropane upon the SCC in toad bladder expressed in percentage of the SCC in the control bladder.[2] The probability of significant differences at 30 minutes between control and cyclopropane 10 per cent was $0.01 > P > 0.001$; cyclopropane 30 per cent $0.01 > P > 0.001$; and cyclopropane 60 per cent $0.02 > P > 0.01$ (see text).

72 hours, and intracardially 24 hours prior to sacrificing them. Isolated bladders from 9 of these were analyzed for content of epinephrine and for norepinephrine.[7] * For comparison the catecholamines were also measured in bladders of 9 control toads. Bladder halves from reserpine-treated toads were exposed to cyclopropane 10, 30, or 60 per cent, with 5 bladders in each group.

(2) Fifteen isolated bladder halves were divided into three groups and treated with l-epinephrine 1×10^{-4} μg./ml., 3×10^{-4} μg./ml., or 1×10^{-3} μg./ml. alone in the bathing solution for 15 minutes, and afterwards in each instance exposed to additional treatment with cyclopropane 5, 10, and 30 per cent consecutively, each concentration for 15 minutes. Five bladders were treated only with cyclopropane.

(3) Nine groups of 5 bladders were treated with phenoxybenzamine 10 μg./ml., 50 μg./ml., or 100 μg./ml.; or phentolamine 25 μg./ml., 50 μg./ml., or 100 μg./ml. in the bathing solution. When the SCC had remained stable for 5 minutes all the bladders were exposed to cyclopropane 10, 30, and 60 per cent, each concentration for 15 minutes. Five bladders were treated only with cyclopropane.

(4) A comparable number of bladders was similarly treated with cyclopropane and a series of β-blockers, pronethalol in a concentration range from 1 to 100 μg./ml., propranolol 0.1 to 100 μg./ml., and an experimental compound MJ-1999 from 100 to 1,000 μg./ml.

The drugs used were: Reserpine (Sandril, Eli Lilly and Company); l-epinephrine (Adrenalin Chloride, Park, Davis and Company); phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline and French Laboratories); phentolamine mesylate (Regitine, CIBA Pharmaceutical Company); pronethalol hydrochloride (Alderin, Imperial Chemical Industries Limited); propranolol hydrochloride (Inderal, Ayerst Laboratories); and 4-(2 isopropylamine-1-hydroxyethyl) methanesulfonamilide hydrochloride (MJ-1999, Mead Johnson Laboratories).†

In all instances of treatment *in vitro*, the pharmacologic agents were added to the bath on both sides of one bladder half, while the other half, in a different bath, was untreated and served as the control. Cyclopropane in oxygen was delivered from a Foregger anes-

* Doctor Aaron H. Anton, Associate Professor, Department of Anesthesiology, College of Medicine, University of Florida, did the catecholamine analyses.

† We wish to thank these pharmaceutical houses for the supply of drugs for this study.

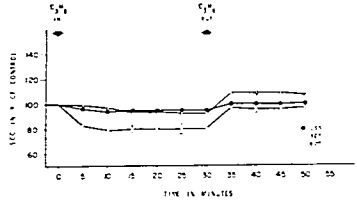

FIG. 2. The effect of cyclopropane upon the SCC in toad bladder from toads pretreated with reserpine. Only with cyclopropane 60 per cent was the depression of SCC below baseline significant ($P < 0.05$) (see text).

thesia machine and bubbled through the chamber; cyclopropane was always preceded and followed by oxygen 100 per cent. Changes in oxygen concentration have previously been shown not to have a significant effect upon the SCC.[2] The concentration of cyclopropane in the gas mixture was determined at 5-minute intervals by means of an "F and M" gas chromatograph.

During the control period SCC values ranged from 18 to 184 (mean 52.0) microamperes in the control bladders, and from 22 to 162 (mean 56.6) microamperes in the test bladders. If the SCC in this period remained below 15 microamperes, the bladder was rejected. The readings in the test bladders were expressed as a percentage of the SCC in the control bladders at the same time, and are presented as such in all figures.

Student's paired *t*-test was applied to all deviations from the baseline of the control. In one instance (fig. 4) de Jonge's test for increasing trend (8) was used.

Results

The results are shown in figures 2 through 6, where each curve represents the mean value of 5 experiments and brackets indicate ±1 standard error of the mean. Three batches of 3 pooled bladders from untreated toads were found to contain epinephrine from 0.412 to 1.116 (mean 0.834) μg./g. and norepinephrine from 0.028 to 0.061 (mean 0.042) μg./g. The same number of bladders from reserpine-treated toads contained epinephrine from 0.026 to 0.057 (mean 0.037) μg./g. and norepinephrine from 0.026 to 0.041 (mean 0.032) μg./g.

Figure 2 shows the effect of different concentrations of cyclopropane in the bladders

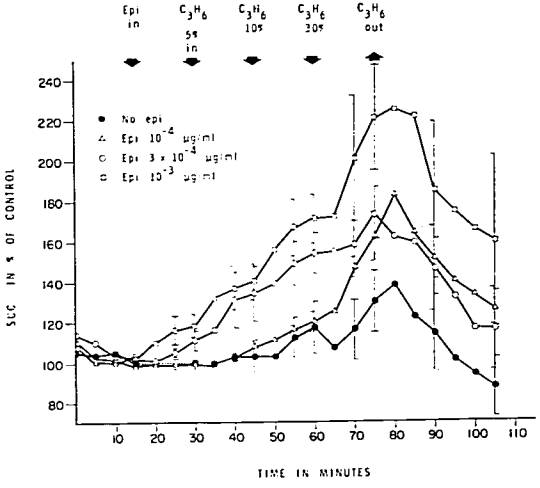

FIG. 3. The effect of epinephrine and cyclopropane alone and together upon the SCC in toad bladder. Note that the highest epinephrine dose (1×10^{-3} μg./ml.) without cyclopropane gave a significant ($P < 0.05$) increase of SCC over the control (see text).

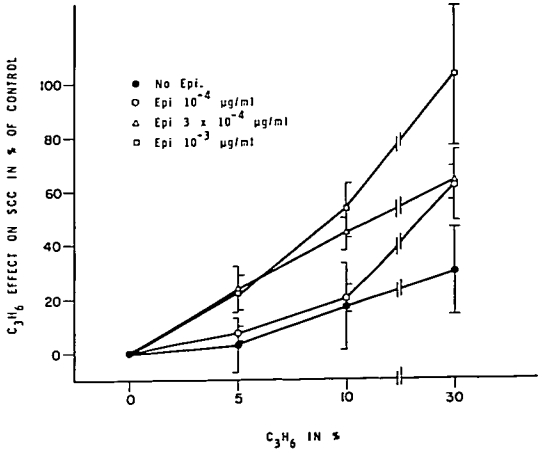

FIG. 4. The effect of cyclopropane upon the SCC in toad bladder with and without epinephrine. The cyclopropane values are the same as in figure 3, but the peak response to each epinephrine concentration given alone was used as the new baseline. Testing for a dose dependent epinephrine effect on the response to cyclopropane with de Jonge's rank test gave $P < 0.05$ (see text).

pretreated with reserpine. Here cyclopropane gave a dose dependent depression of the SCC. The effect was reversible upon discontinuation of the gas.

In figure 3 it is seen that different concentrations of epinephrine in the bath gave a dose dependent stimulation of the bladder. The addition of increasing concentrations of cyclo-

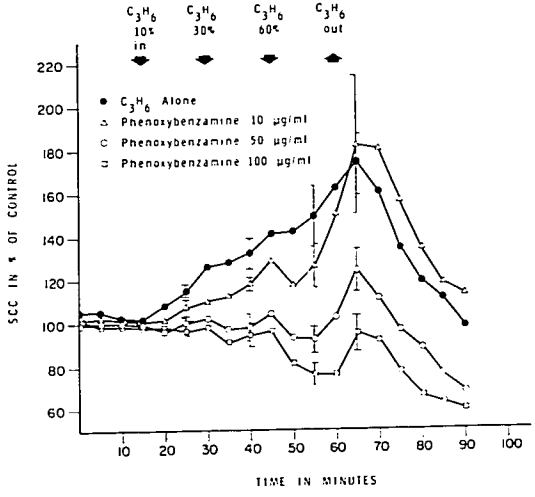

FIG. 5. The effect of cyclopropane upon the SCC in toad bladder with and without phenoxybenzamine. The probability of a significant mean difference between control and all three cyclopropane concentrations without phenoxybenzamine was $P < 0.03$. At cyclopropane 60 per cent with phenoxybenzamine 100 ug./ml. $P < 0.02$ (see text).

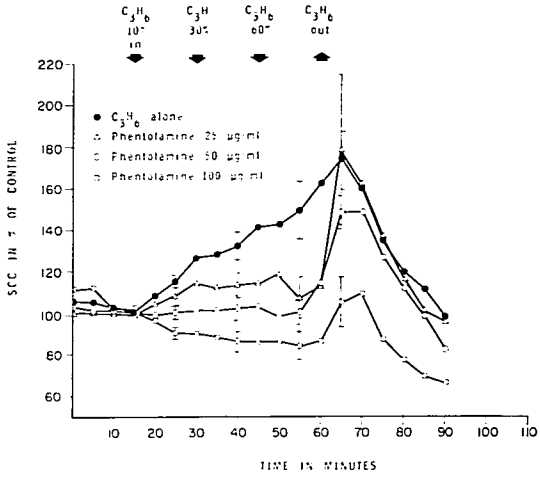

FIG. 6. The effect of cyclopropane upon the SCC in toad bladder with and without phentolamine. The values for cyclopropane alone are the same as in figure 5. The probability of a significant mean difference between control and cyclopropane 30 per cent with phentolamine 100 ug./ml. was $P < 0.05$ (see text).

propane stimulated the SCC further and, beyond the added stimulations of epinephrine and cyclopropane, when these agents were given alone. Discontinuation of the gas reversed the effect.

Figure 4 shows the effect of different concentrations of epinephrine upon the response of the bladder to cyclopropane. The data are the same as in figure 3, but the peak response to each concentration of epinephrine alone was used as the new baseline. The application of de Jonge's test for increasing trend[8] gave a value of $P < 0.05$. For this purpose all data collected throughout the experiment as represented in figure 4 were used. Four groups, all receiving increasing concentrations of cyclopropane, gave the following mean rank values: no epinephrine, 1-2-6-7-18; 1×10^{-4} epinephrine, 5-8-9-10-11; 3×10^{-4} epinephrine, 3-12-13-16-17; and 1×10^{-3} epinephrine, 4-14-15-19-20. Accordingly the hypothesis could be accepted that a trend paralled to the increasing epinephrine concentrations existed.

Figures 5 and 6 show that different concentrations of phenoxybenzamine and phentolamine gave rise to a dose dependent inhibition of the effect of increasing concentrations of cyclopropane so that cyclopropane in the presence of the highest concentration of these α-blockers actually inhibited the sodium transport as expressed by the SCC. Discontinuation of cyclopropane was followed by an increased bladder activity, which was related to the concentration of α-blocker employed; with the highest concentration, only a return to baseline was seen; with the lower concentrations a highly significant further increase in activity occurred, before the bladders returned to baseline values.

The same technique was employed to test the effect of three β-receptor blockers upon the response of the bladders to cyclopropane. None of these drugs were found to interfere significantly with the stimulating effect of cyclopropane.

Discussion

A direct relationship between SCC and active sodium transport in toad bladders had been previously established.[6, 9] While epinephrine was found to stimulate sodium transport in frog skin by Ussing [5] and in toad bladder by McClane,‡ Leaf was unable to demon-

‡ McClane, T. K.: Personal communication.

strate such an effect in the bladder.[10] We believe that our more balanced bathing solution containing glucose and adenosine renders the bladder more sensitive to the effect of drugs. This may be an explanation for the discrepancy between Leaf's report and our finding that epinephrine gave rise to a dose dependent stimulation of sodium transport in toad bladder.

Repeated analyses revealed a considerable concentration of catecholamines in the bladders. It is interesting that in contrast to most species,[7, 11] epinephrine was present in toad bladders in a concentration about 20 times that of norepinephrine. A similar ratio for peripheral tissue was also found in the spleen and liver of the frog.[7] Pretreatment with reserpine reduced the concentration of catecholamines in the bladders to insignificant amounts. The dosages of reserpine employed were relatively high. However, the time of treatment was short, half this dose has been used in rabbits without significant adverse effects,[12] and our dose proved necessary for effective reduction of the catechols in toads. The animals appeared to be more quiescent than usual, and some bladders had to be rejected because the spontaneous SCC was too low. In the absence of a significant concentration of catecholamines in the bladder cyclopropane caused a significant dose dependent inhibition of the sodium transport. This finding is of interest in the light of reports that certain parameters of cardiovascular function were depressed more during cyclopropane anesthesia in reserpine-treated than in nontreated dogs.[13, 14]

In figure 3 it is seen that the stimulation after simultaneous administration of epinephrine and cyclopropane far exceeded the estimated additive effect of the two drugs. This point is better illustrated in figure 4 where the peak response to epinephrine alone was taken as a new baseline for the effect of cyclopropane. The important question here was whether or not the presence of epinephrine in the bath enhanced the cyclopropane effect on SCC. Hence, rank analysis testing for a dose dependent trend was employed (8). The analysis suggested the presence of a significant ($P < 0.05$) epinephrine dose-dependent trend. Thus a synergism between cyclopropane and epinephrine, as previously shown for contraction of smooth muscle in the nictitating membrane [4] and aorta,[5, 15] is likely to exist on a cellular level.

In the part of the study concerned with the nature of the receptor sites, it was found that α-blockers inhibited the stimulating effect of cyclopropane, and that in the presence of the highest concentrations of phenoxybenzamine and phentolamine cyclopropane decreased the SCC. The β-blockers had no effect upon the bladder response to cyclopropane. The lower of the three concentrations of phenoxybenzamine and phentolamine employed here relate well to those reported elsewhere.[16, 17, 18] The three compounds pronethalol,[19, 20, 21] propranolol [22, 23] and MJ-1999 [24, 25] were applied in a concentration range from the smallest dose likely to be effective to the highest dose frequently associated with depression.[26] Neither β-blocker was active. Hence, no statement on an interaction of cyclopropane and β-receptors in the toad bladder is possible.

It is postulated that the synergistic effect of epinephrine and cyclopropane upon the sodium transport in toad bladder is transmitted through an α-receptor.

Through the years, scattered reports have associated the effect of catecholamines with changes in ion flux in cells. In 1955 it was speculated that hyperpolarization of the myocardium after sympathomimetic amines was the result of an increased cellular ion transport,[27] and a later suggestion related this to an increased ATP content in the membrane.[28] Increased permeability of the cell membrane to potassium after adrenergic stimulation [29] has been related to α-receptors [30] and to the phosphorylase-activating effects of catecholamines.[31] In a study of the potassium mobilizing action of epinephrine in the cat, it was possible to dissociate the hyperglycemic response from potassium mobilization by the use of phenoxybenzamine, phentolamine and other α-blockers.[32] Epinephrine was thought to stimulate sodium transport in frog skin through an increased passive permeability to sodium ions.[33] In the present study any increase of the SCC must have been the result either of an increased permeability to sodium or an increased release of carrier molecules in

the membrane. Should the mucosal barrier become permeable to potassium, part of the increase in SCC theoretically could be caused by a potassium transport.[34] Further studies are required before catecholamines, active ion transport, and the mechanism of action of cyclopropane can be fully understood.

Diethyl ether and nitrous oxide can also stimulate ion transport in the toad bladder,[2] possibly through a similar interaction with epinephrine. Nitrous oxide has many features in common with cyclopropane, and ether anesthesia is associated with an increased concentration of circulating catecholamines [35] and is modified by sympathetic block [36] and pretreatment with reserpine.[37] At present we propose the following hypothesis as the basis for further studies: Anesthetics may simultaneously stimulate and inhibit ion transport in cell membranes; stimulation may be the result of interaction with the adrenergic system, depression may be a direct effect. Both effects may be inherent in all anesthetics, but depending upon the agent and the concentration one effect may prevail over the other. This may account for certain systemic differences among anesthetics.

Summary

Cyclopropane stimulated sodium transport in toad bladder through a synergistic effect with epinephrine. In the reserpinized bladder or in the presence of α-blockers cyclopropane inhibited the sodium transport.

I thank Doctor J. S. Gravenstein for his valuable advice.

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Drugs

OPIATE ANTAGONISTS Five cases of overdosage of levallorphan are reported when the dosage usually used for nalophine was mistakenly ordered for levallorphan. Respiratory paralysis followed; in three patients, artificial respiration was used, whereas in two patients opiates were used successfully to antagonize the depressant effect of the levallorphan. (*Harder, H. J., and Leutner, V.: Morphine-Antagonists. Quantum et quado? Overdosage—a Contribution to Well-Dosed and Directed Therapy with Antidotes, Der Anaesthesist 15: 279 (Aug.) 1966.*)