Sodium Transport and Anesthetic Requirements in the Toad

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The effects of cyclopropane, nitrous oxide, thiopental, ether, halothane, and methoxyflurane on sodium transport in the isolated toad bladder were studied. All agents affected sodium transport in the same way: stimulation at low concentrations; inhibition at high concentrations. Stimulation may be the result of positive interaction with catecholamines on the serosa, inhibition the result of a muscarinic effect. The balance between stimulation and inhibition of sodium transport in vitro appeared unrelated to the anesthetic effect in vivo. (Key words: Toad; Ion transport; Sodium; Anesthetic requirements.)

In previous studies we have shown that general anesthetics in concentrations used clinically may stimulate or inhibit active sodium transport in the isolated toad bladder.1–4 Thiopental applied to the mucosa inhibited sodium transport, but applied to the serosa stimulated it. After bilateral application of thiopental the net effect was stimulation at lower concentrations and inhibition at higher concentrations.3,4

In the present study we tested the hypothesis that all general anesthetics have dual effects on sodium transport similar to the effects of thiopental. Cyclopropane, nitrous oxide, ether, methoxyflurane, and halothane were selected for testing.

We also wanted to determine whether the effect on sodium transport could be directly related to anesthetic effect. It was necessary, therefore, to establish the minimal anesthetic requirements of the toad for each agent.2

Method

The technique used has been described.2 Bladders from the toad, Bufo marinus, were divided, and each half was placed between two symmetrical halves of a lucite chamber. One half of the bladder was treated, the other half served as the control. Each half of the symmetrical chamber contained 10 ml of the same bathing solution: sodium chloride, 110 mM; potassium chloride, 10 mM; magnesium chloride, 1 mM; calcium chloride, 0.25 mM; NaH2PO4, 0.9 mM; Na2HPO4, 4.3 mM; TRAM, 5.5 mM; HCl, 2.2 mM; glucose, 33.3 mM; adenosine, 2.8 mM; pH was 8.0 and did not change. The experiments were carried out at room temperature. The solution was aerated with oxygen or oxygen and anesthetic on each side of the bladder.

The toad bladder consists of one layer of mucosal cells, connective tissue, and one layer of serosal cells. An adenosine-5'-triphosphate (ATP)/ATP-ase pump5,6 in the serosal aspect of the serosal cells transports sodium from the mucosa to the serosal surface and gives rise to a transbladder potential. An external electromotive force (short-circuiting current or SCC) nullifying the transmembrane potential may be directly related to active sodium transport.5,6 A Beckman expanded-scale pH meter (membrane potential) and a DC microammeter (SCC) were used for continuous monitoring of the SCC. The sodium pump may or may not transport potassium in the direction opposite to the direction of sodium transport.3 However, a mucosal barrier is ordinarily impermeable to potassium, which diffuses back into the toad so that no net transport of potassium takes place. So long as this barrier to potassium is intact, the sodium pump is electrogenic. We found the barrier to be intact in the presence of high concentrations of thiopental.2

The anesthetic gases and vapors were delivered to the chamber in oxygen from an anesthesia machine. Changes in oxygen concentration from 20 to 100 per cent do not affect SCC. The anesthetic concentrations were determined in the gas phase by gas chromatography in an "F and M" flame ionization unit. Thiopental was pipetted into both halves of
the chamber. This did not cause measurable changes in pH. Cyclopropane, nitrous oxide, thiopental, ether, and halothane were studied in concentrations beyond those previously reported.\textsuperscript{1,2} In addition, the effect of methoxyflurane was tested.

Concentration ranges wider than those described here were tested in pilot studies. Those concentrations that best delineated dose-response curves for stimulation and inhibition of sodium transport were selected for the actual experiments.

After mounting, the bladders were allowed to equilibrate for 30 minutes before the anesthetic was added. Administration of the anesthetic gases and vapors was discontinued when SCC stabilized at a new level, and the bladder was allowed to recover before it was discarded. When SCC in any half-bladder remained below 15 \textmu m, or when SCC did not stabilize during the first 30 minutes after mounting, the bladder was discarded as unsuitable for the study.

The readings in \textmu m were expressed as percentages of the readings in the untreated control half. Most control half-bladders were stable, but

\textbf{Table 1. Effects of Nitrous Oxide on Sodium Transport in the Toad Bladder (Below 1 atm\textsuperscript{4}) and in Frog Skin (Above 1 atm, borrowed from Gottlieb and Savran\textsuperscript{2}) at Equilibrium}

<table>
<thead>
<tr>
<th>N\textsubscript{2}O Tension (atm)</th>
<th>Effect on Na\textsuperscript{+} Transport (Per Cent Change)</th>
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<tr>
<td>0.3</td>
<td>+18.2 ± 4.5\textsuperscript{*}</td>
</tr>
<tr>
<td>0.8</td>
<td>+39.5 ± 12.4\textsuperscript{*}</td>
</tr>
<tr>
<td>6.8</td>
<td>−4.8</td>
</tr>
<tr>
<td>10.2</td>
<td>−63.2\textsuperscript{*}</td>
</tr>
<tr>
<td>14.2</td>
<td>−93.1\textsuperscript{*}</td>
</tr>
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\textsuperscript{*} Difference from untreated control tissues significant at \( P < 0.05 \).

even slightly unstable bladders can be used as controls since SCC changes in paired, untreated half-bladders always parallel each other. Results are given as means ±1 standard error of at least five experiments. Student's \( t \) test was used to assess significance of the differences between test and control values.

\textbf{Results}

Figures 1 through 5 and table 1 show the effects of six general anesthetics on SCC. With each agent, at least one low concentration pro-

\textbf{Fig. 1. Effect of cyclopropane on SCC in the toad bladder, in percentage of untreated control. Values are means of at least five experiments ±SE.}
In the experiments with cyclopropane, ether, halothane, and methoxyflurane, discontinuation of anesthetic administration was followed by cessation of the stimulating or inhibiting effect. However, after discontinuation of stimulating cyclopropane and methoxyflurane concentrations, SCC always increased further for 10–20 minutes before returning toward baseline. Nitrous oxide stimulation had a similar pattern. Generally, stimulation took longer to wear off than inhibition.

Table 2 shows minimum alveolar anesthetic concentrations (MAC) in volumes per cent for the vapors and gases in the toad; for thiopental the minimal blood concentration (MBC) is given in mg/100 ml. MBC is a less precise measurement than MAC. In figure 6 the effect on SCC in the toad bladder is compared with minimal anesthetic requirements in the toad. The anesthetic concentrations are arbitrarily expressed as multiples of minimal anesthetic requirements (MAR = MAC or MBC). It is evident that with cyclopropane and nitrous oxide maximal stimulation of SCC took place at concentrations higher than 3 MAR, with thiopental and ether it took place around 2 MAR, and with methoxyflurane and halothane, below 1 MAR (see discussion). Maximal stimulation was followed by dose-related depression as the concentrations were increased. Cyclopropane appeared to be the most potent stimulator of SCC, and it stimulated SCC over the widest concentration range.
Halothane appeared to be least potent in this respect.

Discussion

Each of the six general anesthetic agents tested increased SCC in low concentrations and inhibited SCC in high concentrations. Nothing in this study invalidates the assumption that changes in SCC paralleled changes in sodium transport across the membrane.6, 8

The results with nitrous oxide above one atmosphere were borrowed from Gottlieb and Savran,19 who used a different technique and tested frog skin rather than toad bladder. However, amphibian sodium transport was measured, and earlier studies have shown that there is no fundamental difference between sodium transport in frog skin and that in toad bladder.6, 8 Pilot experiments in our laboratory showed that the two transport systems react similarly to anesthetics. Gottlieb and Savran19 found that at about 7 atm nitrous oxide had no effect on sodium transport, but at higher pressures it produced dose-related
Table 2. Minimal Anesthetic Requirements (MAR) of the Toad at Room Temperature, as Determined by a Standard Stimulus

<table>
<thead>
<tr>
<th></th>
<th>CCl₄ (Vol Per Cent)</th>
<th>N₂O (Vol Per Cent)</th>
<th>Ether (Vol Per Cent)</th>
<th>Halothane (Vol Per Cent)</th>
<th>Methoxyflurane (Vol Per Cent)</th>
<th>Thiopental (mcg/100 ml Blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAR</td>
<td>9.0 ± 0.21</td>
<td>82.2 ± 0.1</td>
<td>1.64 ± 0.04</td>
<td>0.07 ± 0.01</td>
<td>0.22 ± 0.01</td>
<td>2.83 ± 0.45</td>
</tr>
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inhibition. We found that at pressures lower than one atmosphere nitrous oxide stimulated sodium transport. These two areas of the curve describing the effects of different nitrous oxide concentrations on sodium transport in amphibian animals look much like the curve describing the effect of cyclopropane in the toad bladder. This suggests similar relationships between transport and anesthetic effects for nitrous oxide and cyclopropane (fig. 6).

Earlier experiments with cyclopropane and thiopental showed that stimulation of sodium transport in the toad bladder depends on the presence of endogenous or exogenous catecholamines. Simultaneous application of a catecholamine and an anesthetic actually resulted in positive interaction between the two compounds. With thiopental this interaction could be elicited on the serosa only. Thiopental inhibited sodium transport on the mucosa only, an effect which may have been brought about by decreased membrane permeability to sodium. Thiopental lends itself particularly well to studies of this nature since in these short experiments the effect of application on one side was unrelated to the effect on the other side.

In the present study cyclopropane and methoxyflurane both were associated with greater stimulation of sodium transport after discontinuation of the anesthetic than during its ad-

Fig. 6. The relationship between effect on sodium transport in vitro and anesthetic effect in vivo in the toad. The anesthetic effect is expressed in multiples of 1 minimal anesthetic requirement (MAR) as determined by response to a standard stimulus.
administration. With every agent stimulation seemed to wear off more slowly than inhibition after discontinuation. We interpret this to indicate that all six anesthetics in principle affected sodium transport in the same way: simultaneous stimulation and inhibition of sodium transport, stimulation being the result of interaction with endogenous catecholamines (epinephrine) in the serosa.\(^2\) inhibition of the result of decreased mucosal permeability to sodium. Once elicited, stimulation lasted longer than inhibition. The net effect after bilateral application depended on the concentration, so that low concentrations stimulated and high concentrations inhibited transport.

Comparison of the transport effects with the anesthetic effects showed no direct correlation, however. Thus, our results seem to be compatible with the following conclusions:

1) The six anesthetics tested, and maybe all general anesthetics, stimulate sodium transport at relatively low concentrations.
2) At relatively high concentrations the same agents inhibit sodium transport.
3) Stimulation of transport is an expression of positive interaction with catecholamines.
4) Inhibition is an expression of a depression that overshadows the concurrent stimulation.
5) The balance between stimulatory and inhibitory effects on sodium transport in vitro appears unrelated to the anesthetic effect in vivo.

These conclusions are suggested for the toad. Extension to other species is not justified at this time. When the anesthetics tested are ranked according to their ability to stimulate sodium transport in the toad bladder, the usual clinical evaluation of the cardiovascular effects of the same anesthetics in man is brought to mind. Stimulation of smooth muscle with cyclopropane and inhibition with halothane\(^1\) could be cited. The possibility of a relationship in this area deserves further study.

References

Drugs

CEPHALEXIN Cephalexin, a new cephalosporin antibiotic, provided effective treatment of 17 patients with bacterial pneumonia. Twelve patients had pneumococcal pneumonia, while five patients had pneumonia caused by gram-negative organisms. Toxic effects included: cosinophilia, SGOT elevations, diarrhea, and rash. No difficulty with the use of cephalaxin was encountered in patients allergic to penicillin. (Fass, R. J., and others: Cephalexin—A New Oral Cephalosporin, Amer. J. Med. Sci. 259: 187 (March) 1970.)