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Effects of Methoxyflurane and Two Metabolites on Sodium Transport in the Toad Bladder

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Sodium transport in toad bladders was reversibly decreased by 0.2–1.0 vol per cent methoxyflurane. Depression of sodium transport was also produced by .1–10.0 mM NaF, a metabolite of methoxyflurane. This depression was irreversible at 10.0 mM NaF. Another possible metabolite, oxalate, increased sodium transport, whereas the chelating agent EDTA decreased it. Depression of active renal sodium reabsorption is accompanied by an increase in water excretion, *e.g.*, high urinary output. The methoxyflurane metabolite, inorganic fluoride, was shown to cause depression of sodium transport. Thus, the results are consistent with the postulate that high-output renal failure after methoxyflurane is caused by its metabolite, fluoride. (Key words: Ions, sodium: transport; Anesthetics, volatile: methoxyflurane; Biotransformation: methoxyflurane.)

METHOXYFLURANE ANESTHESIA sometimes causes renal dysfunction characterized by increased serum creatinine, increased urinary output, and decreased urinary osmolarity.[1] This nephrotoxicity is a result of biotransformation.[2] The metabolites of methoxyflurane include inorganic fluoride and oxalic acid, both of which may be nephrotoxic.[3] Fluoride is known to inhibit renal Na^+–K^+-dependent ATPase,[4] which could explain high-output renal failure through inhibition of Na^+ and water reabsorption in the renal tubules, but methoxyflurane itself also inhibits active Na^+ transport.[5] Oxalic acid, on the other hand, is more likely to be associated with oliguric renal failure.[6]

The present study was undertaken to assess the effects of methoxyflurane, fluoride, and oxalic acid on active Na^+ transport. The toad bladder was chosen as the model because it transports sodium from mucosa to serosa and contributes to sodium and water conservation in the animal in a kidney-like fashion and because anesthetic requirements of methoxyflurane in the toad have been estimated.[7]

The toad bladder consists of one layer of mucosal cells, connective tissue, and one layer of serosal cells. An ATP/ATPase pump[8,9] in the serosal aspect of the mucosa transports sodium from the mucosal to the serosal surface and gives rise to a transbladder potential.[10] Active sodium transport is directly related to an external electromotive force (short-circuiting current or SCC) nullifying the transmembrane potential.[8,11]

Method

Toads (*Bufo marinus*) were pithed. The bladder was removed from each, divided, and mounted between two symmetrical halves of a lucite chamber. One half of each bladder was treated; the other served as control. Each half of the chamber contained 10 ml of the same bathing solution: NaCl, 110 mM; KCl, 10 mM; $MgCl_2$, 1 mM; $CaCl_2$, 0.25 mM; NaH_2PO_4, 0.9 mM; Na_2HPO_4, 4.3 mM; tris, 5.5 mM; HCl, 2.2 mM; glucose, 33.3 mM; adenosine 2.8 mM. The experiments were carried out at room temperature; *p*H was 7.8 and did not change. The solution on both sides of the bladder was aerated with oxygen or oxygen and anesthetic. A Beckman expanded-scale *p*H meter (membrane potential) and a DC microammeter were used for continuous monitoring of short-circuiting current.

Three groups of five bladders each were treated with methoxyflurane 0.2, 0.5, or 1.0 vol per cent for 50 minutes, whereupon the bladders were permitted to recover for 30 minutes. The anesthetic was delivered in oxygen from an anesthesia machine, concentrations were monitored by gas chromatography.

Sixteen bladders were treated with increasing concentrations of sodium fluoride. The

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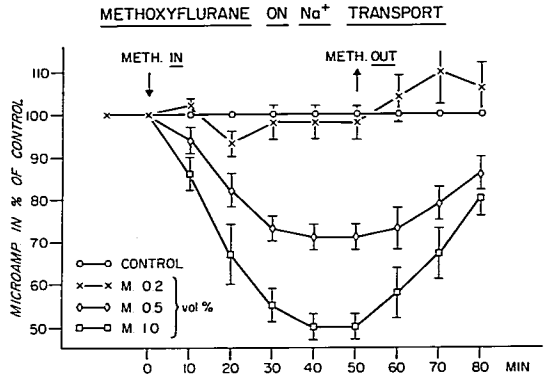

FIG. 1. Effects of three concentrations of methoxyflurane (Meth. or M) on sodium transport in toad bladders. Values are means ± SE for five bladders.

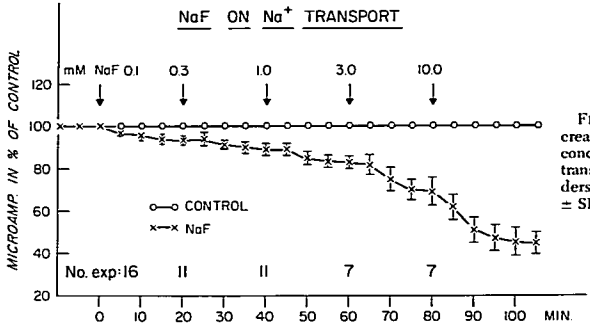

FIG. 2. Effects of increasing sodium fluoride concentrations on sodium transport in 16 toad bladders. Values are means ± SE.

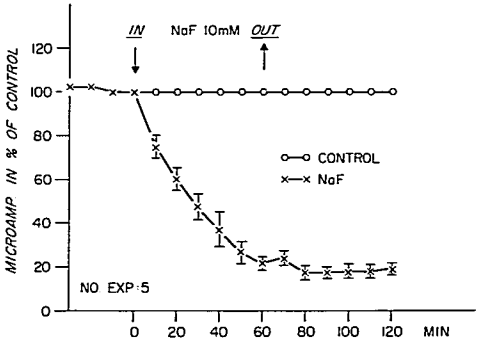

FIG. 3. Effect of 60-minute exposure to 10.0 mM sodium fluoride on sodium transport in five toad bladders. Values are means ± SE.

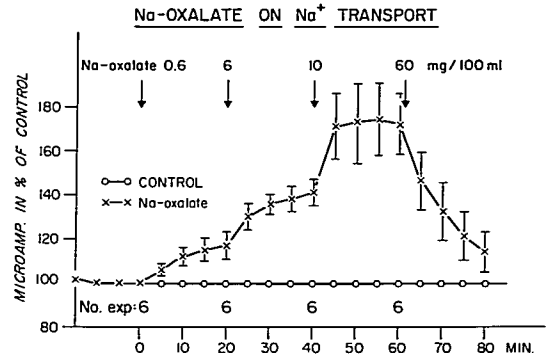

FIG. 4. Effects of increasing sodium oxalate concentrations on sodium transport in six toad bladders. Values are means ± SE.

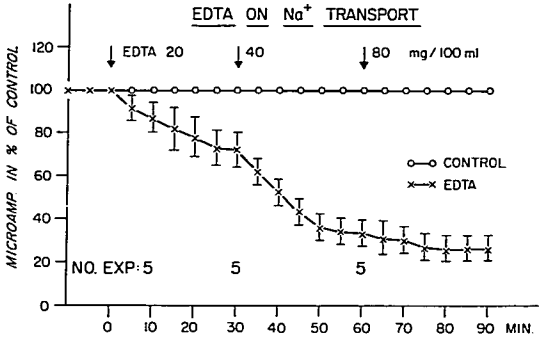

FIG. 5. Effects of increasing concentrations on ethylenediaminetetraacetic acid (EDTA) on sodium transport in six toad bladders. Values are means ± SE.

concentrations were 0.1, 0.3, 1.0, 3.0, and 10.0 mM. The effect of each dose was monitored for 20 minutes before the next increment was added.

Five bladders were treated with sodium fluoride, 10.0 mM, for 60 minutes, whereupon the fluoride solution was removed and rapidly replaced with a drug-free solution. Recovery was monitored for 60 minutes.

Six bladders were treated with increasing concentrations of sodium oxalate: 0.6, 6.0, 10.0, and 60.0 mg/100 ml. For comparison with the effect of oxalate, another five bladders were treated with EDTA (ethylenediaminetetraacetic acid), 20, 40, and 80 mg/100 ml.

Any bladder that showed a short-circuiting current of less than 15 μamp at the beginning of an experiment was discarded, and any experiment with an unstable control was discontinued. The results in the test bladder, expressed as percentages of control, are presented as means ± 1 SE. Student's t test was used to assess significance.

Results

Methoxyflurane, 0.2–1.0 vol per cent, progressively decreased sodium transport as shown in figure 1 ($P < .01$). When the anesthetic was discontinued after 50 minutes, transport values promptly approached control.

Incremental doses of sodium fluoride progressively decreased sodium transport (fig. 2). Even the smallest concentration tested, 0.1 mM, after 20 minutes had a slight but consistent and significant effect ($P < .01$), and the highest concentration, 10.0 mM, decreased the short-circuiting current to 40 per cent of control in the same period of time.

Fluoride, 10.0 mM, in a different set of experiments, reduced the short-circuiting current to 20 per cent of control in 60 minutes; following removal of the drug from the bathing solution the bladder failed to show any recovery when observed for another 60 minutes (fig. 3).

Sodium oxalate in increasing concentrations had the exact opposite effect, progressively stimulating transport up to a concentration of 10 mg/100 ml ($P < .01$, fig. 4). Sixty mg/100 ml steeply decreased the short-circuiting current. Oxalate binds calcium, but the calcium chelator EDTA was found to inhibit rather than stimulate sodium transport. Figure 5 shows the effects of increasing EDTA concentrations, *e.g.*, 20–80 mg/100 ml, which progressively decreased transport ($P < .01$).

Discussion

A direct relationship between the short-circuiting current and active sodium transport has been established in the untreated bladder[8] and the treated bladder.[12]

In this study we found that methoxyflurane and one of its metabolites, fluoride, decreased the short-circuiting current and thus sodium transport in a dose-related progression. The effect of methoxyflurane was reversible, and transport returned towards normal when the anesthetic was discontinued. This effect was produced by methoxyflurane at and above the minimal anesthetic requirements of methoxyflurane in the toad, 0.22 vol per cent.[7] Smaller concentrations have previously been shown to stimulate sodium transport.[5] Tissue tensions higher than those corresponding to equilibrium at inhaled concentrations of 1.0 vol per cent are probably rarely found during clinical methoxyflurane anesthesia. We therefore tend to consider this reversible effect on sodium transport to be of relatively minor importance for renal function.

Fluoride, a metabolite of methoxyflurane,[13] also decreased active sodium transport. This effect was elicited in a dose-related fashion at incremental concentrations ranging from 0.1 to 10.0 mM in the solution. One dose of fluoride, 10.0 mM, virtually abolished transport in an hour (short-circuiting current = 20 per cent of control), and this effect proved irreversible within the limits of the study.

Mazze *et al.*[3] found a mean peak serum inorganic fluoride concentration of 0.2 mM in patients with renal dysfunction after methoxyflurane anesthesia, and others have reported urinary concentrations as high as 0.9 mM.[14] Mazze *et al.*[15] also showed a positive correlation between serum fluoride levels and polyuria in rats, and Frey *et al.*[2] presented evidence that this is also true in man. Fluoride, therefore, appears likely to be the causative factor in polyuric post-methoxyflurane renal failure. Fluoride has been shown to inhibit renal Na^+-K^+-activated transport ATPase from guinea pigs[16] and rats,[4] in addition to the generally accepted inhibitory effects of glycolysis. In toad bladder a prolonged block of active sodium transport seems invariably associated with degenerative changes in the cells.[17]

Oxalate, somewhat surprisingly, stimulated sodium transport in a concentration range of 0.6 to 10.0 mg/100 ml. Patients with clinical signs of renal dysfunction after methoxyflurane have been shown to excrete as much as 300 mg of oxalate in 24-hour urine samples.[19] This stimulating effect on sodium transport is not compatible with post-methoxyflurane polyuric syndrome, but could be related speculatively to anuric renal failure following acute oxalic acid intoxication,[6] and to oliguric failure after the combination of methoxyflurane and tetracycline.[18]

Since oxalic acid reduces the concentration of ionized calcium in biologic media, we also tested the effect of a calcium chelating agent, EDTA. EDTA, however, had an effect opposite to that of oxalic acid on sodium transport and decreased the transport in comparable concentrations. Thus, we are unable at this time to explain the effects of oxalic acid and EDTA on sodium transport in toad bladders.

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Metabolism

HYPEROSMOLAR NONKETOTIC DIABETIC COMA It would appear that a diabetic patient not receiving insulin who is given a course of propranolol may develop hyperosmolar nonketotic coma. A 46-year-old man treated for one year with hydralazine, 200 mg, and propranolol, 240 mg, daily, was admitted in coma with dehydration. Ketonemia was absent. He was treated with insulin and hypotonic infusions, and subsequently given tolbutamide, 1 g daily. Several months later he had a similar episode. The authors then studied the development of hyperglycemia in this patient, first without propranolol therapy and then during propranolol therapy. Without insulin or propranolol and on a free diet, the patient's blood sugar rose to 630 mg/100 ml in five to six weeks; this was accompanied by 2 + ketonemia and ketonuria and an elevation in serum free fatty acids and osmolarity. The patient was treated with insulin for a few days until all values returned to normal, then was given oral propranolol 240 mg daily. In eight weeks the blood sugar rose to 930 mg/100 ml and serum osmolarity to 335 mOsm/kg. Ketosis was absent and free fatty acids rose only slightly. It is suggested that propranolol may act in two ways. Free fatty acid mobilization from adipose tissue or lipolysis is a beta-adrenergic effect which is blocked by propranolol. Insulin release is suppressed by alpha-adrenergic stimulation, and this is enhanced by beta blockade. *(Podolsky, S., and Pattavina, C.: Hyperosmolar Nonketotic Diabetic Coma: A Complication of Propranolol Therapy. Metabolism 22:685–693, 1973.)*