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Effects of Substance P on Na and K Transport in Perfused Main Submandibular Duct of Rat (41927)

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Abstract. Substance P (2 and 4 μg/kg · min, iv) caused an inhibition of net efflux of Na and net influx of K in perfused main excretory duct of rat submandibular gland. These effects could not be blocked by atropine sulfate. The data suggest that substance P receptors are present in the duct cells and play a role in the regulation of transductal electrolyte transport.

Substance P (SP) is an undecapeptide present in mammalian central nervous system and in peripheral nerve endings supplying several tissues, including the salivary glands. A number of studies show that SP can elicit salivary secretion from parotid (1–4), and submandibular glands (4–6). It is also possible that SP may play a role in the regulation of electrolyte transport in the salivary duct system.

The main excretory duct of submandibular when perfused through its lumen exhibits appreciable electrolyte transport with no significant changes in water flux (7–10). Moreover, transport by ductal epithelium can be regulated by stimulation of autonomic nerves (11–13), by autonomic drugs (9, 10, 14), and by other drugs as well (15–17). The perfused main duct is thus a useful system to examine drug effects on electrolyte transport, and, therefore, the present study was undertaken to investigate the effects of SP on Na and K transport. This study is of especial pertinence since the peripheral action of SP has thus far not been completely delineated.

Materials and Methods. Long–Evans male rats, 3–4 months of age, were anesthetized with pentobarbital sodium (50 mg/kg, ip). Details of the surgical procedures have been described earlier (7–9, 13). In brief, one main excretory duct of the submandibular was cannulated as its oral opening, using PE-10 tubing beveled at its tip, for collection of perfusate samples while its hilar end was cut and cannulated with PE-10 tubing pulled to a tip diameter 70–100 μm for perfusion.

The experimental animals were divided into two groups: in the first, SP was administered iv at dosages of 2 and 4 μg/kg · min; in the second, atropine sulfate, a parasympatholytic drug, was administered at 5 μg/kg, ip, followed 20 min later by administration of SP at the same dosages.

It has been shown that the primary saliva contains plasma-like electrolyte concentrations and their concentrations are modified by processes of reabsorption and secretion during passage along the salivary duct system (18, 19). Therefore, the perfusion solution used contained 144 m*M* Na, 5 m*M* K, 122 m*M* Cl, and 27 m*M* HCO_3. The perfusion rate was set at 1 μl/min. At least a 20-min period of perfusion was allowed to stabilize the animals, and samples collected during this period were discarded. The control microliter-perfusate samples were then collected for Na and K analysis by flame photometry (Instrumentation Labs, Inc., Model 143). SP was subsequently administered. Three to four samples were collected for each maneuver, and a mean value was calculated. Thus, 9 to 12 min were consumed for each maneuver.

In some experiments, a trace amount of [^{3}H]methoxyinulin was added into the perfusion solution for the determination of water flux. The inulin ratio and net electrolyte flux have been calculated for the entire duct as in previous reports (11–13). Net electrolyte flux has been calculated for the entire duct according to the formula

$$J = V(C_i - C_f)I$$

where J is the net electrolyte flux (neq/min · duct), V the perfusion rate (nl/min), C_i and C_f the electrolyte concentration (neq/nl)

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in the perfusion fluid and the collected perfusate, respectively, and I the inulin ratio. A positive sign indicates efflux from the lumen and a negative sign indicates influx.

In analysis of data, values in the text and table are represented as means ± SE. Control values were compared with experimental data within the same animal by paired Student's t test. Values were considered to be statistically significant if P values were less than 0.05.

Results. One to two minutes after administration of SP (2 μg/kg · min) a copious flow of saliva from parotid and submandibular was observed, and when the dosage was increased to 4 μg/kg · min salivary flow from both glands became even more copious. The dosage levels needed to elicit secretion were thereby established. Before infusion of SP, net efflux of Na was 26.1 ± 2.0 and net influx of K was 22.2 ± 2.6 neq/min · duct. Infusion of SP (2 μg/kg · min) caused a decrease ($P < 0.05$) of about 30% in net efflux of Na and 20% in net influx of K (Table I). When SP was increased to 4 μg/kg · min the net fluxes of Na and K were even more markedly decreased ($P < 0.01$), and net fluxes of Na and K were 43 and 39% of control, respectively (Table I).

In a second group, net efflux of Na and net influx of K were 27.8 ± 2.1 and 27.5 ± 2.6 neq/min · duct, respectively (Table I). Atropine sulfate (5 μg/kg, ip) alone did not alter the transepithelial fluxes of Na and K.

TABLE I. EFFECTS OF SUBSTANCE P AND EFFECTS OF ATROPINE SULFATE PRIOR TO THE ADMINISTRATION OF SP ON TRANSDUCTAL ELECTROLYTE TRANSPORT

	neq/min · duct	
Perfusion period	Net Na efflux	Net K influx
Group 1, control (6)	26.1 ± 2.0	22.2 ± 2.6
SP 2 μg/kg · min, iv	18.3 ± 2.3*	16.5 ± 2.6*
SP 4 μg/kg · min, iv	15.0 ± 1.1**	13.5 ± 3.0**
Group 2, control (4)	27.8 ± 2.1	27.5 ± 2.6
Atropine 5 μg/kg, ip	26.5 ± 1.3	27.5 ± 2.2
SP 2 μg/kg · min, iv	19.5 ± 2.4*	20.3 ± 2.6*
SP 4 μg/kg · min, iv	15.0 ± 1.4**	16.3 ± 2.7**

Note. Values are means ± SE. Numbers in parentheses refer to number of animals. In group 2, atropine sulfate was administered 20 min prior to collection of the samples; SP was subsequently administered. *$P < 0.05$ and **$P < 0.01$. Efflux = electrolyte moves from lumen to interstitial fluid. Influx = electrolyte moves into the lumen.

Moreover, the atropine did not block SP-induced changes of transepithelial electrolyte transport (Table I).

Discussion. It has been shown that SP evokes a large but transient secretion of saliva, with the composition qualitatively similar to that induced by parasympathomimetic agents (4, 6). However, while Na concentration of SP-evoked saliva shows increases with increasing flow rate, it is higher than that of parasympathetically evoked saliva when flow rates are comparable (4, 6). These findings suggested that SP may have effects at both acinar and ductal cells. Present data also show effects of SP on ductal cells since administration of this peptide caused inhibition of net fluxes of Na and K. These effects are qualitatively similar to those caused by carbachol (9), high doses of isoproterenol (9), and direct stimulation of the sympathetic nerve (11). Such data suggest that SP as well as cholinergic and adrenergic receptors are present on duct cells. Thus, SP receptors play a role in regulation of transepithelial electrolyte transport, and may have a role in determining the volume and composition of salivary secretion.

Present data do not provide an explanation for the inhibitory effect of SP on Na and K transport. Although it has been demonstrated that this peptide increases membrane K conductance in acinar cells of salivary glands (3, 21), it is unlikely that it increases K conductance in duct cells since present data show that SP inhibits net K efflux. Moreover, in myenteric neurons, SP does in fact decrease membrane K conductance (20). These findings suggest that SP may decrease K conductance in the duct cells, and Na conductance as well. However, it is also possible that SP may interfere with the Na:H antiport or K:H antiport (22).

Since the inhibitory effects of SP on ductal electrolyte transport are not prevented by atropine, it is concluded that SP receptors alone are involved in mediating the effects. On the other hand, the SP-induced secretion from whole salivary glands probably involves SP activation of cholinergic as well as SP receptors (2, 4), since atropine partially inhibits the secretion (2, 4). Furthermore, it was recently shown that SP decreases the secretory response to acetylcholine and isoproterenol but does not modify the binding

of specific ligands to glandular autonomic receptors (6). It was concluded that the inhibitory effect of SP may involve some steps in stimulus secretion coupling localized beyond receptor activation. A modulating action of SP on autonomic effects may also be involved (6).

Leeman and Hammerschlag (1) found that SP-induced salivation from parotid gland cannot be blocked by atropine, phenoxybenzamine, or propranolol. On the other hand, Inoki *et al.* (2) and Yu *et al.* (4) reported that atropine significantly inhibits SP-induced salivation but shows no effect on amylase secretion. The discrepancy between these studies is probably due to the fact that Leeman and Hammerschlag did not use purified SP and their method of collecting saliva would not readily show a decrease in salivary flow.

In view, however, of the conflicting reports on effects of atropine on SP actions, it may be necessary to reexamine effects of atropine at different dosages, although atropine does block cholinergic muscarinic actions at very low dosage by any route of drug administration (23).

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