^{42}K spaces in submandibular gland of early postnatal rats: effects of carbachol and transport inhibitors

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Summary. Equivalent spaces of ^{42}K were measured in fragments of the submandibular gland of 1-, 7-, 14- and 21-day-old and adult rats in the absence and presence of carbachol and the transport inhibitors ouabain and furosemide. The results indicate that the ^{42}K space was increased by carbachol in an ouabain-sensitive manner at all ages studied and that part of the secretagogue-stimulated K uptake occurred by way of a furosemide-sensitive transport system in the latter part of the postnatal period and in the adult.
Key words. Submandibular gland; postnatal development; ^{42}K spaces.

We have recently demonstrated that fragments of the submandibular gland of early postnatal and adult rats show K^+ efflux and reuptake when exposed to acetylcholine in a superfusion chamber. The reuptake of K^+ was sensitive to both ouabain and furosemide[1] but the extent of furosemide-sensitive reuptake varied with age, becoming progressively larger as age increased, while ouabain-sensitive uptake was already active in 1-day-old rats[1]. These results suggested that there is a temporal dissociation in the appearance of two major ion transport systems in salivary glands during postnatal development. An ouabain-sensitive Na, K pump is present and quite active soon after birth, while a furosemide-sensitive co-transport system believed to be involved in saliva formation[2-4] does not become completely functional until later in the postnatal period. Equivalent spaces of ^{86}Rb, an isotope employed as a marker of K movements[5], have been used to characterize the effects of cholinergic stimulation on K transport in salivary gland slices[6]. It was thus shown that carbachol enhanced ^{86}Rb uptake (influx) and that this effect was blocked by ouabain[6]. In view of our findings with superfused submandibular fragments of rats of different postnatal ages, K spaces were measured in this study in submandibular gland fragments of postnatal and adult rats with the aid of the isotopic tracer ^{42}K. These spaces were measured both in the absence and presence of carbachol and of ouabain, which inhibits the Na, K ATPase, or of furosemide, a loop diuretic which blocks a cation/anion co-transport system which appears to transport Na, K and Cl into salivary and other cells[7,8].

Methods. Pregnant female rats of the Sprague-Dawley strain were obtained from Sasco Laboratories (St. Louis, Missouri) approximately 1 week before term. The pups were removed at 1, 7, 14 and 21 days of age and were anesthetized with i.p. pentobarbital (1–3 mg/100 g b.wt). The submandibular glands were removed, placed in a small volume of an enriched Krebs-Ringer bicarbonate solution and cut into small fragments with a sharp scalpel blade. The fragments were thoroughly mixed and divided into equal portions (approximately 50 mg) which were placed in nitrocellular tubes containing 1 ml of medium for a 10 min equilibration (pre-incubation) period. At the end of this period, the fragments were washed once in fresh medium and then placed in 1 ml of the final incubation solution. Both the pre-incubation and final incubation media contained (in mM): NaCl, 118; KCl, 3.0; $CaCl_2$, 2.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 2.8; inosine, 10; adenine, 0.5; nicotinamide, 10; β-hydroxybutyrate, 5; were adjusted to pH 7.4 and were aerated with a 95% O_2-5% CO_2 gas mixture at 37°C. The fragments were incubated in the absence or in the presence of carbamylcholine (2×10^{-5} M) for 2 min in normal medium and in medium containing 1 mM of either ouabain or furosemide. 0.1–0.2 µCi/ml of ^{42}K was then added to the various systems and 5 min later the tissue was removed, weighed and homogenized in a glass homogenizer with Teflon pestle. Samples of the homogenate (100 µl) were placed in scintillation fluid and counted for radioactivity in a scintillation counter. Similar samples of the incubation medium were similarly analyzed and equivalent ^{42}K spaces were calculated by dividing CPM/g tissue over CPM/ml and expressed as ml/g. Mean values and SD were calculated from the data and statistical comparisons were made using Student's t-test.

Results. Table 1 shows the results of the measurement of ^{42}K spaces in the resting (unstimulated) gland fragments from rats of the different ages. These data show that the ^{42}K space is lower in 1-day-old rats, but becomes similar from 7 days of age on. Ouabain has only a small effect in this resting ^{42}K space in 1- and 7-day-old rats (p values > 0.5) but reduces it 21–29% in gland fragments of 14- and 21-day-old rats and of adults ($p < 0.01$). By contrast, furosemide has essentially no effect (1–6% change) in the resting ^{42}K space of fragments studied at the different ages ($p > 0.005$).

Table 2 summarizes the data obtained when the fragments were exposed to carbachol. Comparison of the ^{42}K spaces measured in the absence of transport inhibitors in the resting (table 1) and stimulated (table 2) fragments indicates that carbachol increased the ^{42}K space at all ages studied. This increment was not age-dependent and varied between 20 and 55% but was highly significant at all ages ($p < 0.01$). The data shown in table 2 also indicate that the increase in the ^{42}K spaces was inhibited by both ouabain and furosemide. The inhibition by ouabain was between 38% (in 1-day-old rats) to 46–53% in older animals and was statistically significant ($p < 0.001$) in all cases. The inhibition by furosemide varied according to age, being 10% in 1-day-old-rats (borderline significance, $p = 0.05$), approximately 20% in 7- and 14-day-old rats ($p < 0.01$) and 26–27% in 21-day-old and adult rats ($p < 0.001$).

Discussion. The ^{42}K spaces in submandibular gland fragments of adult rats which were obtained in this study are similar to those previously reported in the rat parotid gland[6]. As in the case of the latter tissue, the resting ^{42}K space is increased by cholinergic stimulation, an effect which is inhibited by ouabain. This suggests that ouabain-sensitive K uptake by the Na, K pump is enhanced by stimulation of cholinergic receptors, i.e., that the

Table 1. ^{42}K spaces in resting (unstimulated) submandibular fragments of postnatal and adult rats

Age (days)	N	^{42}K space (ml/g)	Space in the presence of 1 mM ouabain (ml/g)	Space in the presence of 1 mM furosemide (ml/g)
1	5	0.95 ± 0.08	0.90 ± 0.02	0.90 ± 0.04
7	6	1.16 ± 0.06	1.05 ± 0.07	1.18 ± 0.09
14	5	1.23 ± 0.04	0.89 ± 0.03	1.19 ± 0.04
21	4	1.26 ± 0.07	0.92 ± 0.07	1.15 ± 0.04
Adult	5	1.23 ± 0.05	0.87 ± 0.08	1.20 ± 0.09

Values are means ± SD of the mean. N = Number of experiments.

Table 2. ^{42}K spaces in submandibular fragments of postnatal rats after exposure to 10^{-5} M carbamylcholine

Age (days)	N	^{42}K space (ml/g)	Space in the presence of 1 mM ouabain (ml/g)	Space in the presence of 1 mM furosemide (ml/g)
1	6	1.30 ± 0.11	0.81 ± 0.03	1.16 ± 0.06
7	5	1.57 ± 0.04	0.84 ± 0.03	1.27 ± 0.11
14	6	1.42 ± 0.09	0.67 ± 0.07	1.13 ± 0.11
21	7	1.43 ± 0.05	0.73 ± 0.05	1.05 ± 0.05
Adult	5	1.91 ± 0.05	0.94 ± 0.04	1.41 ± 0.05

Values are means ± SD of the mean. N = Number of experiments.

activity of the pump increases in the stimulated tissue. Our data reveal several characteristics of this K uptake during postnatal glandular development and also indicate that it occurs in part by a furosemide-sensitive transport system in later stages of postnatal life and in the adult animal.

In resting (unstimulated) tissue, K uptake appears to be smaller and not inhibited significantly by ouabain in the first few days of life. This suggests that the ouabain-sensitive pump has a relatively low resting activity during this early period of postnatal gland development. Morphologically, the rat submandibular gland consists mostly of terminal tubular and proacinar cells at this period[9-11] and it is possible that these precursor cells have a pump with a resting activity lower than that of the fully differentiated salivary cells of later developmental stages. Our data indicate, however, that the ^{42}K space (i.e., K uptake) was significantly enhanced by carbachol and that this effect was uniformly inhibited by ouabain at all ages studied. This suggests that the Na, K pump can be activated by cholinergic stimuli from very early phases of postnatal development, as previously suggested[1,12,13].

Our results also indicate that part of the secretagogue-stimulated K uptake occurs by way of a furosemide-sensitive transport system after approximately 3 weeks of life (table 2). This transport system does not seem to be active in the absence of stimulation at any of the ages studied and although present in the glands of early postnatal animals does not become fully responsive to the cholinergic stimulus until later in development. These findings support the view that there is a temporal dissociation in the appearance of two major ion transport systems in the rat submandibular gland during postnatal development[1]. A secretagogue-activated Na, K pump is present from the immediate postnatal period, while a furosemide-sensitive transport system which moves Na, K and Cl across the salivary cell membrane does not become fully responsive until later in development. As both the Na, K pump and the furosemide-sensitive co-transporter are thought to participate in the formation of saliva[2-4,7], this dissociation is likely to influence the ability to secrete during the early phases of postnatal gland development[14].

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Restraint stress induced changes in rat liver and serum metallothionein and in Zn metabolism

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Summary. 24 h of a psychogenic stress (restraint) caused a strong increase of liver metallothionein (MT) levels. 3 h of stress were sufficient to induce an increase in liver MT, measured 21 h later, but the increase was much lower than in continuously restrained rats. Stress induction of liver MT was not due to food deprivation, since rats deprived for 24 h showed lower MT levels than stressed ones. Zn on MT presented the same qualitative but not quantitative pattern of response as MT protein. Liver cytosolic Zn was increased by restraint in spite of their being no decrease in serum Zn. Any treatment altered serum MT. Liver and serum MT were not correlated. The present results demonstrate that basically psychogenic stresses increased liver but not serum MT levels. No positive evidence for a relationship between corticosterone secretion and MT induction was found.
Key words. Metallothionein; pituitary-adrenal; zinc; restraint stress; copper.

Metallothioneins (MT) are low molecular weight, cysteine-rich, heavy metal-binding proteins. They are distributed among several species and organs, in which their synthesis is induced by the metal ions to which they bind, i.e. Cd, Zn and Cu[1]. Although its physiological function(s) remains to be established, it has been hypothetized that MT is related mainly to Zn and Cu metabolism[2]. In addition, it has been reported that some stress stimuli increase liver[3-10] and serum[8] MT. However, previous studies of the effect of these stimuli on MT had serious limitations: a) These stimuli could be considered mainly as physical stressors. Since it is believed that the term stress must be restricted to those stimuli which have mainly a psychological component[11], the effect of a psychogenic stress on MT levels remained to be established. b) No attempts were made to determine whether or not a continuous exposure to the stress stimulus for several hours was necessary to induce MT synthesis. c) No attempts were made to separate the effect of these experimental manipulations per se from their effect on food intake. This seems important since these stimuli might alter food intake[12], and deprivation increases hepatic MT[8]. d) Liver MT levels were measured indirectly using different methods[13-19]. It is therefore difficult to compare quantitatively the results obtained by different authors and their relevance with regard to stress-induced MT synthesis. Furthermore, because of the lack of specifity and sensitivity of these methods MT levels in serum were not measured in most studies. e) Glucocorticoids have been related to MT regulation both in vivo[20-23] and in vitro[24-26] studies, but reports are also conflicting[3,27]. To give insight into the relationship between glucocorticoids and MT, pituitary-adrenal hormones and MT were studied jointly in normal and stressed rats. Recently, a highly specific and sensitive radioimmunoassay method for MT has been developed[28-30]. It is the purpose of the present work to study the effect of a psychogenic stress (restraint) on liver and serum MT as well as on pituitary-adrenal hormones and reevaluate the relationship between MT and Zn metabolism during stress.

Materials and methods. Animals. Female Sprague-Dawley rats (85 ± 6 days old) were housed in groups of four per cage in a controlled room (light on 7–19 h, temperature 22 °C, white noise constant) for at least one week before starting experiments. Food and water were available ad libitum.

Procedure. At 09.00 h the animals were randomly assigned to the following experimental groups: A) Control: rats left undisturbed in the animal house and killed at the same time as re-