The present results can be summarized as follows. 1) ANF produced a dose-dependent relaxation of isolated monkey, rabbit and dog internal carotid arteries. 2) The relaxations were not affected by the pretreatment with 10^{-7} M propranolol. 3) There was a marked species difference in the ANF-induced relaxation. The response was greatest in the monkey arteries and least in the dog arteries. 4) The relative sensitivities to ANF, which were represented as the ratios of the 4×10^{-8} M ANF-induced relaxations to the maximum relaxations developed by sodium nitroprusside, suggested the same tendency: greatest in monkey and least in dog.

The ANF is a peptide hormone which has a potent vasodilator activity as well as a natriuretic action[6]. Effects of ANF on rat and rabbit aorta, mesenteric and renal arteries are well studied[3,4]. Garcia et al.[4] suggested that there was a marked heterogeneity in the response of different vascular preparations to ANF.

Fujioka et al.[7] showed that i.v. infusion of 3 µg/kg ANF caused a significant fall in mean arterial pressure with no change in cerebral blood flow in anesthetized rat, implicating a fall in blood flow resistance in the brain. No report, however, has been found about the direct effect of ANF on isolated cerebral arteries except that by Faison et al.[5], who demonstrated a slight ANF-induced vasodilation in rabbit basilar artery.

Responsiveness of cerebral arteries to vasoactive substances is extremely different from that of extracranial arteries; 5-hydroxytryptamine is a more potent vasoconstrictor agent than noradrenaline in the cerebral arteries[8]. Responses of internal carotid artery to vasoactive agents are similar to those of cerebral arteries[9,10]. Thus, our results suggest that ANF may have a potent relaxant action on the monkey cerebral arterial smooth muscles. There is a marked species differences in the ANF-induced relaxation of the cerebral arteries. A low concentration (4×10^{-9} M) of ANF relaxed the monkey internal carotid arteries by almost 100%, the relative relaxation being expressed as percent of submaximal contraction developed by 10^{-6} M noradrenaline; and a higher concentration (4×10^{-8} M) of ANF produced further relaxation in those preparations. In contrast to the monkey strips, an ANF concentration a hundred times higher, 4×10^{-7} M, was needed to produce near 100% relaxation in rabbit arteries. Furthermore, the same concentration of ANF caused less relaxation (62.5 ± 3.6%) in the dog internal carotid arteries. Our results also show that the ANF-induced relaxation is not mediated by a beta-adrenergic receptors. This is consistent with the results obtained in the rat and rabbit strips[4]. Further study will be needed to clarify the mechanism of the ANF-induced relaxation.

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Na, K ATPase activity during early postnatal development of the rat submandibular gland

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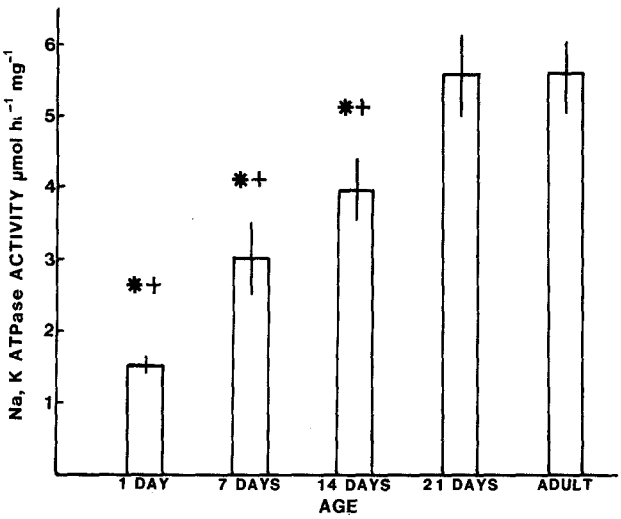
Summary. The activity of the ouabain-sensitive Na, K ATPase was measured in membrane fractions of the submandibular gland of 1-, 7-, 14- and 21-day-old rats. This activity increased with age and reached adult levels by 21 days.
Key words. Submandibular gland; Na, K ATPase; postnatal development.

Slices of the rat submandibular gland exposed in vitro to appropriate secretagogues show a net release of K^+ which is the result of passive K^+ efflux balanced by K^+ reuptake[1,2]. The latter is inhibited by ouabain, which suggests that it occurs primarily by activation of a Na, K ATPase. Previous studies indicated that this ouabain-sensitive K^+ uptake was high in submandibular slices of newborn (i.e., 1-day-old) rats and became smaller as the age of the animals increased[3,4]. This finding suggested that the activity of the Na, K ATPase responsible for K^+ uptake changes as the gland matures during postnatal development. However, this K^+ uptake was measured in those studies under conditions which would markedly enhance pump activity, such as after a previous incubation in K^+-free medium, which results in K^+ loss and in Na^+ entry, i.e., in the type of change in cell electrolytes which would activate the Na, K pump. It was also observed that slices of 1-day-old rats also had a larger preceding passive efflux of K^+ under the experimental conditions used and that, at other ages, K^+ reuptake was also proportional to the preceding efflux. Thus, it was not clear if the differences in K^+ uptake observed at different postnatal ages were due to a change in the activity of the Na, K ATPase or to artifacts arising from the experimental conditions employed. The purpose of this study was, therefore, to directly measure Na, K ATPase at different postnatal ages, by using membrane fractions of the submandibular gland of 1-, 7-, 14- and 21-day-old rats. The results were compared with those obtained in fully mature glands of adult rats.

Methods. Pregnant Sprague Dawley rats were obtained from Sasco Laboratories approximately 1 week prior to delivery. The pups were maintained with the mothers and were removed when they reached the ages indicated above. They were anesthetized with i.p. injections of sodium pentobarbital and the submandibular glands were excised, minced on ice and homogenized at 4°C in 10 times (v/w) the volume of a buffer containing 25 mM imidazole, 300 mM sucrose, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 2 mM dithiothreitol (DTT) in a Polytron homogenizer. An aliquot of the homogenate was removed for DNA analysis. The rest of the homogenate was centrifuged at $1000 \times g$ for 10 min and the resulting supernatant was then centrifuged at $100{,}000 \times g$ for 30 min. The resulting pellet was resuspended in 25 mM imidazole to a final concentration of 1–3

mg protein/ml. Measurements of total and ouabain-sensitive ATPase activity were carried out in triplicate at 37 °C. The assay mixture contained 500 µl of 12.5 mM imidazole, 100 mM NaCl, 10 mM KCl, 3 mM $MgCl_2$ and 3 mM ATP plus 30–50 µg of membrane protein. In some tubes 1 mM ouabain was added to the mixture to measure the ouabain-sensitive portion of the ATPase. The difference between total ATPase (measured in the absence of ouabain) and the portion inhibited by the glycoside was taken as the Na, K ATPase. The reaction was terminated by the addition of 6% sodium dodecyl sulfate (SDS) and the Pi liberated was measured by the method of Fiske and Subbarow[5]. Protein in the same membrane fractions used for the ATPase assay was measured by the Lowry method[6]. Analysis of DNA in aliquots of tissue homogenates was carried out by a modification of the method of Dische[7]. Briefly, 0.5 ml of 0.6 N HaOH was added to 0.5 ml of tissue homogenate and heated 60 min at 37 °C. Then 1 ml of ice cold 1 N $HClO_3$ was added and the mixture was incubated at 4 °C for 10 min. The mixture was centrifuged at 1000 × g for 10 min and the pellet was washed once with 0.2 N $HClO_3$ and resuspended in 0.5 ml of 0.5 N $HClO_3$. The DNA standard (1 mg/ml) and the tissue pellets were hydrolyzed at 90 °C for 15 min then centrifuged at 1000 × g for 10 min. The resulting supernatant and DNA standard were transferred into 0.5 N $HClO_3$ to a final volume of 500 µl. 1 ml of 3% diphenylamine containing 5 µl/ml of 1.6% acetaldehyde was added to each tube and incubated overnight at room temperature. Readings were made at 600 nm in a Beckman DU spectrophotometer.

Results. The results (expressed per mg protein) are shown in the figure. Na, K ATPase activity in the submandibular gland progressively increased from 1.5 ± 0.32 µmol h^{-1} mg^{-1} at 1 day post partum to 5.7 ± 0.79 µmol h^{-1} mg^{-1} at 21 days of age. Enzyme activity at 21 days of age was not significantly different from that in gland preparations from adult animals (5.7 ± 0.48 µmol h^{-1} mg^{-1}) but it was significantly smaller (p values < 0.01) at all other ages studied. Statistically significant differences were also ascertained between 1 and 21 days when Na, K ATPase activity at a given age was compared with that observed in the subsequent age group. Total ATPase activity measured in the absence of ouabain was 32.6 ± 0.77 µmol h^{-1} mg^{-1} at 1 day of age, increased to 37–38 at 7 and 14 days and was 50.4 ± 3.1 µmol h^{-1} mg^{-1} at 21 days of age. In the adult, fully mature gland this total ATPase activity was 67.6 ± 2.5 µmol h^{-1} mg^{-1}. Similar



Na^+, K^+ ATPase in membrane preparations (100,000 × g fraction) of the submandibular gland of rats at different postnatal ages. Values are means ±SE of the mean and are based on 4–8 experiments. *, Significantly different from adult value. +, Significantly different from subsequent age group.

DNA content and Na, K ATPase activity in the developing submandibular gland

Age of the animals	N	DNA content (gland homogenate) mg/ml	Na, K ATPase activity µmol h^{-1} mg DNA^{-1}
1	8	0.25 ± 0.02*	6.3 ± 1.2*
7	4	0.39 ± 0.02*	8.4 ± 2.0
14	6	0.45 ± 0.02*	9.0 ± 1.2
21	3	0.44 ± 0.03*	11.7 ± 1.3
Adult	4	0.52 ± 0.02	10.9 ± 0.9

Values are means ±SE of the mean. * Significantly different from adult value.

results were obtained for Na, K ATPase when the values were expressed per mg of DNA (table). Thus, Na, K ATPase activity almost doubled between 1 and 21 days of age, with intermediate values being observed at the intervening ages which were studied. The difference in enzyme activity between 1- and 21-day-old animals and between the former and adult was significant ($p < 0.01$). After 7 days, these differences were not statistically significant (table). As shown in the table, DNA content also increased with age and was twice as much in the glands of 21-day-old rats as it was in those of 1-day-old animals. The values were statistically different at all ages studied when compared to those in adult tissues.

Discussion. The results show that the resting activity (i.e., in the absence of stimulus) of the ouabain-sensitive Na, K ATPase increases with advancing postnatal age in the rat submandibular gland. The data indicate that the increase in enzyme content parallels the increase in DNA which occurs at this period of postnatal development. The latter is likely to reflect the progressive proliferation of cells (mostly acinar cells) which is known to occur during this period[8]. Son et al.[9] previously reported that the activity of K^+-dependent phosphatase (which represents the dephosphorylation process of the Na, K ATPase) also increased in the rat submandibular gland between birth and 20 days of age. Similarly, Na, K ATPase activity increased from birth in rat kidney plasma membranes and was onyl 38% of the adult activity in the newborn[10]. In our study, Na, K ATPase activity in glands of 1-day-old rats was 26% of that in adults. The age-related increase in Na, K ATPase activity observed in this study needs to be reconciled with previous findings that K^+ uptake (which is thought to occur primarily by way of the ouabain-sensitive Na, K pump) becomes progressively smaller as the gland matures during postnatal development[3,4]. First, the enzyme activity measured in this study was that activity shown in the absence of stimulus. Our previous study of ^{42}K spaces in gland slices from rats of different postnatal ages also indicated that this resting activity of the Na, K pump was lower in younger animals (i.e., 1–7 days old), but that it could be activated significantly by stimulation even at this early age[12]. On the other hand, the K^+ uptake studied previously was that observed either after cholinergic stimulation or under other experimental conditions leading to activation of the pump[3,4]. Together, these findings suggest that although less enzyme is present in the resting (unstimulated) gland of younger animals, it can be maximally activated by the proper stimuli. This significant activation at early ages could be due, at least in part, to the fact that stimulation causes a larger prior efflux of K^+ in the glands of younger animals[3,4]. Thus, although less enzyme is present, its activation would be relatively greater than in the glands of older animals because of the more pronounced electrolyte changes (i.e., K^+ loss) induced by stimulation. Second, K^+ uptake can take place by mechanisms other than the Na, K pump as the gland matures during the postnatal period. Our recent studies have indicated that part of the secretagogue-induced K^+ uptake occurs by way of a furosemide-sensitive transport system, which is not very active in the glands of younger animals and only becomes fully responsive at 3 weeks of age[11,12]. Thus, the relative contribution

of the Na, K pump to K^+ uptake seems to become less as the gland matures, despite the fact that more enzyme is available. Third, the extent of stimulation-induced K^+ uptake may depend, in addition to the availability of Na, K ATPase and of the diuretic-sensitive transport system, on changing metabolic conditions as the gland matures during postnatal development. The finding that the ratio of K^+ uptake to the preceding efflux in perfused slices exposed to carbamylcholine decreases with increasing postnatal age[11] could be the result of changes in metabolic pathways leading to the activation of K^+ transport systems during maturation. This possibility requires further investigation. Finally, it is possible that enzyme recovery in the crude membrane preparations shows age-related differences which contribute to the findings of this study.

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Entrainment of human circadian rhythms by artificial bright light cycles

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Summary. Artificial bright light cycles (LD 8:16) of about 5000 lux during the light period were applied to two subjects in a temporal isolation unit, who had shown free-running circadian rhythms in sleep-wakefulness and rectal temperature. The circadian rhythms were successfully entrained by the artificial light cycle, but the phase relation of the rhythms to the light cycle was substantially different between the two subjects. The result indicated that the artificial bright lights are able to reset human circadian rhythms.
Key words. Human circadian rhythm; entrainment; artificial bright lights; temporal isolation.

In spite of many observations which have indicated a zeitgeber effect of light cycles for circadian pacemakers in mammalian species, the role of light in the human circadian system has been a matter of debate[1,2]. Previously, Aschoff and his coworkers[3] reported an entraining effect of artificial light cycles on the human sleep-wake cycle and rectal temperature rhythm under temporal isolation. The intensity of lights during the light period was 300 lux and the subjects were allowed to use a supplemental night lamp during the dark period. They found that the circadian rhythms entrained to an artificial light cycle, not only of a 24-h period but also of a period other than 24 h. There was also a range of entrainment. This artificial light cycle, however, was complemented by a gong signal given at regular intervals, which alerted the subject to urine micturition and test sessions. Several years later, they realized that this regular gong signal had itself acted as a strong zeitgeber[4]. Without gong signals, the human circadian rhythms did not entrain to artificial light cycles.

More recently, Czeisler and his coworkers[5] reported similar experiments and concluded that a light-dark cycle alone could be an effective zeitgeber. They imposed artificial light cycle with total darkness during the dark period, and showed apparently entrained circadian rhythms. But the work was criticized by others[2,6], who pointed out that when it was completely dark, the subject was immobilized and forced to sleep. This effect of complete darkness was distinguished from the entraining effect on the circadian pacemaker, and termed a behavioral effect. Recently, bright lights stronger than 2500 lux have been reported to suppress the nocturnal elevation of the blood melatonin level in humans, which was not affected by lights of an intensity lower than 500 lux[7]. This finding suggested that the sensitivity of human circadian system, not of the visual system, to light was very low as compared with that of other mammals, and also suggested that bright lights similar to natural day light might be able to reset human circadian rhythms. Later it was found that bright lights, in contrast to dim lights, could increase the range of entrainment of human circadian rhythms under temporal isolation[6], and produced phase-delay shifts of body temperature rhythm independent of the time of the sleep-wake cycle[8]. In the present study, we report entrainment of human circadian rhythms by artificial bright light cycles in two subjects under temporal isolation.

Two young male subjects lived alone in a temporal isolation unit[9] for 2 weeks without knowledge of time. The unit was made by reconstructing rooms on the 2nd floor of a hospital dormitory. The unit consists of a living room, a kitchen, a toilet and shower room and an anteroom through which the unit is connected to the outside. The walls, ceiling and floor were shielded with sound-proof materials. The anteroom is equipped with a double lock system, so that the subject does not encounter any experimenter. There are two kinds of illumination in the unit. One is provided by ordinary lights of about 300–500 lux at the level of subject's head, located in the living room, kitchen, and toilet and shower facilities. The other is an array of bright lights, located on the ceiling of the living room, whose light intensity is 5000 lux at the level of desk surface underneath the equipment, and 500 lux on the pillow on the bed. The subject was allowed to use the lights of lower intensity at any time, but control of the bright lights was possible only from outside the unit. Ventilation of the unit was continuously in operation. The room temperature was controlled by the subject himself. Meals were supplied from outside without announcement. Food was placed on a refrigerator in the anteroom 3 times a day at irregular intervals. In addition, the subject was able to cook by himself. Communication with the subject was performed by letter through the anteroom. Sleep-wakefulness cycles were recorded by the subject. They were instructed beforehand to turn off the bed lamp before falling asleep and turn it on immediately after waking. The signal was fed into a recorder located outside the unit.