ureter (fig. 1). The ureteral contractions induced by rat urine were abolished by pre-incubation of the enzyme with kallikrein antiserum (fig. 2).

Discussion. The antibodies obtained against purified rat urinary kallikrein proved to be homogenous when analyzed by immunoelectrophoresis. Since the antibodies blocked the blood pressure lowering effect of kallikrein it may be suggested that they bound to the active site of the enzyme, although it is also possible that they prevented its binding with kininogen.

Rat urine induces contractions of the isolated rat ureter[2]. The same effect can be evoked by purified urinary kallikrein. This, together with the inhibition of the contractions to rat urine obtained with kallikrein antiserum supports our previous suggestion that the stimulatory effect of urine is caused by its kallikrein content. Kininogen is also present in urine[7]. Thus, locally released kinins could facilitate urine flow through the ureters. The stimulatory effect of kinins upon the ureteral musculature in vivo was described several years ago[8].

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cGMP stimulates active K^+ uptake in rat submandibular slices

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Summary. The 8-bromo derivative of cGMP was found to stimulate the ouabain-sensitive uptake of K^+ and to reduce the net release of K^+ induced by acetylcholine in rat submandibular gland slices incubated in vitro.

Parasympathomimetic stimulation of the rat submandibular gland causes both the release of K^+ in vitro[1-4] and the formation of cyclic GMP[5,6]. Both responses are dependent on the availability of external Ca^{++} and appear to occur in essentially similar time courses[5,6]. The net release of K^+ is the result of 2 opposing and almost simultaneous mechanisms, a passive efflux and an active reuptake. The latter seemingly depends on the activation of an ouabain-sensitive Na^+, K^+, ATPase[1,3,4]. The role of cyclic GMP in K^+ release from salivary glands is, however, still unclear. One study on the parotid gland suggested a role for the cyclic nucleotide in K^+ release[7] while others have questioned the involvement of cGMP in this process[6,8-10]. The latter view is based upon experimental observations showing that 3-isobutyl-1-methylxanthine, an inhibitor of phosphodiesterase, enhanced the effect of agonists on cGMP but not on the release of K^+. However, these experiments were routinely carried out in the presence of ouabain, which inhibits the Na^+, K^+ ATPase presumably responsible for active K^+ uptake. More recent observations have shown, on the other hand, that cyclic GMP stimulates the hydrolysis of p-nitrophenyl-phosphate (pNPP), an alternate substrate for the Na^+, K^+ ATPase[11], in both the salt gland of the duck[12] and in slices of the rat submandibular gland[13]. The present study was carried out, therefore, to assess a possible function of cyclic GMP on the active uptake component of the K^+ release mechanism of the rat submandibular gland.

Methods. Adult, male rats of the Sprague Dawley strain were used in all the experiments. The animals weighed between 200 and 280 g and were fed a standard pelleted diet and water ad libitum. Submandibular glands were excised under pentobarbital anesthesia, separated from the adjoining sublingual gland and rapidly cut into slices of approximately 1 mm^3 in a small quantity of incubation medium which had been bubbled with a 95% O_2-5% CO_2 mixture and warmed to 37 °C for at least 30 min prior to use. Slices from the glands of 4-6 rats were pooled, thoroughly mixed and then divided into approximately equal portions containing 150-200 mg tissue. Each portion was rapidly placed in a nitrocellulose tube containing 2 ml of the incubation medium for a 10-min pre-incubation. Each slice system was then washed with oxygenated, warm medium and then placed in 2 ml of fresh medium for the final incubation. The medium used throughout this procedure was a Krebs-Ringer bicarbonate solution (KRB) enriched with betahydroxybutyrate, adenine, inosine and glucose as previously described[3]. The time when the slices were placed in the final incubation medium was considered as zero time. Secretagogues or other agents were added, alone or in combination, to the slice systems. Aliquots of the medium were subsequently taken at timed intervals

Net K^+ release from submandibular gland slices of control rats

Stimulant	Net K^+ release percent of total (10 min)	N
Acetylcholine	21.0 ± 1.2	6
Acetylcholine + cGMP	6.2 ± 0.2	6
Acetylcholine + MIX	12.9 ± 0.9	6

Experimental details are described in the text. Acetylcholine was added at zero time to the final incubation medium in a dose of 2×10^{-5} M; cGMP was added to a final concentration of 10^{-5} M; 3-isobutyl-1-methylxanthine (MIX) was used in a final concentration of 10^{-5} M. These 2 agents were added 10 min before acetylcholine. Release of K^+ is expressed as the percent of the K^+ content of the slices released after 10 min of incubation. Results are means ± SD.

(usually 2, 5 and 10 min) for the analysis of their K^+ concentration. At the end of the incubation period, the slices were homogenized in a Polytron homogenizer. The K^+ concentration of the aliquots of medium removed in the course of the final incubation and of the slice homogenate was measured in an Instrumentation Laboratories flame photometer with lithium internal standard and the release of K^+ was expressed as a percentage of the K^+ contained in the slices by using a previously described formula[4]. In some experiments the slices were pre-incubated in media modified by the removal if K^+. In experiments involving more than 1 agent, the drugs were added sequentially, one substance 10 min before the 2nd agent (see results, below).

The following agents were used: 1. carbamylcholine; 2. 8-bromo cyclic GMP; and 3. 3-isobutyl-1-methylxanthine. All were purchased from Sigma Chemical Company, St. Louis, Missouri. The doses of these agents used in various experiments are detailed in the results section.

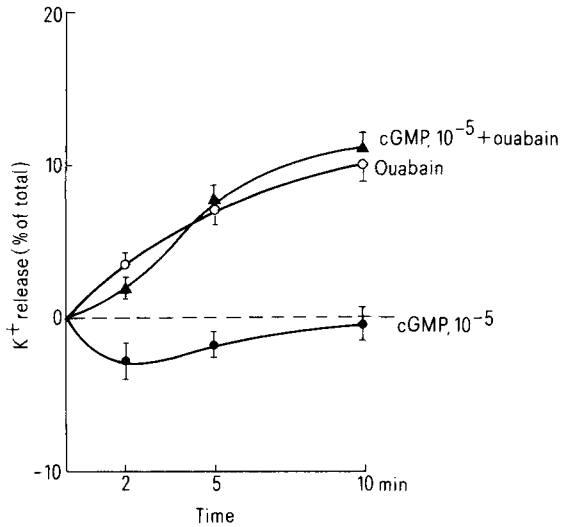

Figure 1. Release of K^+ from submandibular gland slices in the presence of cGMP, ouabain, and cGMP plus ouabain. Tissue slices were incubated in an enriched, oxygenated medium and test substances were added to the incubation medium at zero time to the final concentrations indicated. The results shown are the means of 6 similar experiments. Vertical bars represent ± SEM.

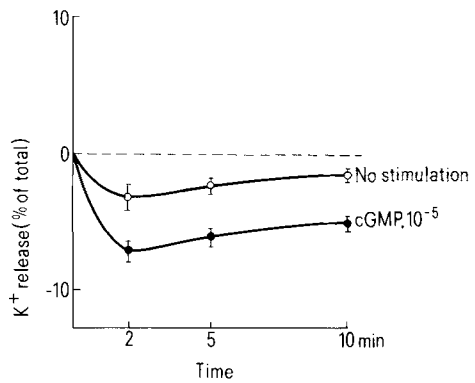

Figure 2. Uptake of K^+ in submandibular slices transferred to K^+-containing incubation medium after a 15-min pre-incubation in K^+-free medium. cGMP was added to the final incubation in 6 experiments, while no drug was added in 6 parallel experiments. Values shown are means of these experiments. Vertical bars represent ± SEM.

Results. Addition of the 8-bromo derivative of cGMP (10^{-5} M) to submandibular slices did not cause an increase but rather a decrease in the net release of K^+ (fig. 1). The negative sign for the K^+ release observed at the various time points shown in the curve illustrating the effect of cGMP were obtained with the formula used to calculate K^+ release[4] and indicate that the K^+ concentration in the medium actually decreased following addition of the nucleotide derivative. Since the slice preparation is a 'closed' system, this suggests that K^+ was taken up into the tissue. After 10 min of incubation, the value obtained with the formula was $-0.10 \pm 0.10\%$ K^+ uptake. Since submandibular slices incubated in KRB medium in the absence of cGMP or stimulants show a $6.3 \pm 1.0\%$ basal release of K^+ after 10 min of incubation, the results obtained with 8-bromo cGMP suggest that this nucleotide inhibited this basal leakage of K^+ from the gland slices. At a final concentration of 10^{-8} M, cGMP caused a net release of K^+ of $1.7 \pm 0.3\%$ after 10 min of incubation. This value was significantly lower than that of the basal (unstimulated) release.

Figure 1 also shows that when the nucleotide derivative was added to final incubation media containing 1 mM ouabain, a $11.1 \pm 1.4\%$ passive efflux of K^+ was observed. However, this extent of K^+ efflux was essentially similar to that caused by ouabain alone (fig. 1), which amounted to $10.0 \pm 1.3\%$. These observations suggest that cGMP does not modify the passive K^+ efflux component of the K^+ release mechanism.

Further evidence for the role of cGMP on active K^+ uptake was obtained in experiments in which the 8-bromo derivative of cGMP was added to slices transferred to KRB medium containing 4.5 mEq/l K^+ after pre-incubation in medium containing no K^+. Slices exposed to these incubation conditions actively take up K^+ during the second incubation in K^+-containing medium. This response is illustrated in figure 2 which also shows that this active K^+ uptake ($1.9 \pm 0.4\%$) was significantly enhanced ($4.9 \pm 0.4\%$) when 8-bromo cGMP was added to the medium at the time of transfer to complete KRB.

In addition, the 8-bromo derivative of cGMP significantly reduced the net K^+ release induced from the slices by acetylcholine when the 2 agents were added sequentially to the final incubation medium (table). Thus, acetylcholine alone caused a $21 \pm 0.12\%$ net release of K^+. In the presence of the 8-bromo derivative of cGMP, however, this value decreased to $6.2 \pm 0.2\%$. Since, as shown above, the cGMP derivative did not modify passive K^+ efflux, this reduction in net K^+ release is likely due to an enhancement of the active K^+ uptake component. The effect was indeed blocked by 1 mM ouabain (not shown). The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (MIX) also inhibited the acetylcholine-induced net release of K^+, although to a lesser extent than cGMP (table).

Discussion. The results of these experiments show that cGMP stimulates active K^+ uptake in slices of the rat submandibular gland incubated under appropriate experimental conditions. Both passive K^+ efflux and active K^+ reuptake occur in salivary gland slices upon stimulation of appropriate receptors and the net release of K^+ observed from these preparations is the result of these two opposing and almost simultaneous mechanisms[1,3,4]. The active uptake component of this mechanism can be ascertained more clearly when appropriate modifications are used in the incubation conditions. The effects of 8-bromo cGMP during regular incubation conditions and during conditions modified to enhance active K^+ uptake (by pre-incubation in medium containing no K^+) indicate that the nucleotide enhances this active process. In the absence of ouabain, which inhibits active K^+ uptake[1,3,4], furthermore, cGMP

reduces the acetylcholine-induced net K^+ release, as a result of enhanced K^+ uptake. Stimulation of active K^+ uptake by cGMP seems to be the consequence, furthermore, of a stimulatory effect of the nucleotide on the Na^+, K^+ ATPase responsible for active K^+ uptake, since addition of ouabain prevented the observed stimulation of K^+ uptake by the nucleotide. Shi et al. have shown that cGMP stimulates the hydrolysis of pNPP, a substrate for the Na^+, K^+ ATPase[13]. The mechanism by which cGMP stimulates the Na^+, K^+ ATPase was not directly investigated in our study, but could involve effects on a protein kinase or on a regulatory protein that inactivates the pump mechanism.

Active K^+ uptake by the Na^+, K^+ ATPase is also observed in rat submandibular[4] and parotid[14] slices after stimulation of α-adrenergic receptors. However, a recent study failed to demonstrate an increase in cGMP formation in rat submandibular gland slices following α-adrenergic receptor stimulation[6]. It is possible that active K^+ uptake during the response to α-adrenergic stimuli is mediated by a mechanism which does not involve cGMP. However, differences in the time course of cGMP generation by cholinergic and α-adrenergic stimuli should be considered, since α-adrenergic stimulation is effective in stimulating cGMP formation in other tissues, including the parotid gland[8,9], and it is not clear why the submandibular gland should be an exception. Our results indicate that the function of cGMP in the K^+ release mechanism of the rat submandibular gland is to enhance active K^+ uptake. This function is physiologically important, since recovery of the K^+ lost as a result of the passive efflux induced by receptor stimulation would restore the ionic composition of the salivary cells to the prestimulation levels, and, thus, render them responsive to subsequent secretory stimuli. The activation of the $Na+$, K^+ pump during the response to secretagogues will also result in the active extrusion of Na^+ from the salivary cells and contribute, therefore, to the formation of saliva. The energy required for the ionic movements responsible for fluid secretion may derive from that stored in the Na^+, K^+ gradient across the cell membrane.

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Extracellular volume, electrocardiogram and anion distribution in hibernating hamster ventricle[1]

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Summary. The intracellular Cl concentration, $[Cl]_i$, was found to be significantly larger and the extracellular volume (ECV), much smaller in ventricles of hibernating hamsters as compared to non-hibernators. The decreased ECV in ventricles of hibernators was consistent with an increased R wave component of the ECG, as well as a lower mean fraction tissue water, f_{H_2O}, of these tissues.

It is still uncertain whether Cl ions are distributed passively or actively across the membrane of cardiac muscle cells. It is largely accepted that the Cl concentration in heart muscle exceeds values compatible with a passive distribution[2–4], however, evidence is available which supports a passive Cl distribution in some cardiac tissues[5–7]. This apparent discrepancy in the literature is in part due to the fact that it has been difficult to measure Cl content of muscle cells with sufficient precision[8]. This difficulty rests in the fact that the cellular Cl content and concentration in striated muscles are small relative to the high extracellular content and concentration; and the relatively large statistical dispersion of extracellular space measurements observed when muscles are examined in vitro has led to unacceptable large dispersions when intracellular Cl, $[Cl]_i$, and cellular Cl content are derived by conventional compartmental analysis.

Using improved techniques for measuring Cl content and concentration[8], Golnick et al.[9] recently measured values of $[Cl]_i$ in in situ hamster ventricle which was significantly less than reported earlier for heart muscle, but consistent with the recent observations of Polimeni and Page[6] for rat ventricle. These data would suggest that Cl is passively distributed in the intact rat and hamster ventricle. To further support the idea of a passive distribution in these tissues, it would be desirable to compare our calculated Cl equilibrium potential (derived from the in situ distribution of Cl) to the in situ cardiac membrane potential. These data, however, are difficult to interpret since the distribution of Cl in contracting heart muscle is fixed to a mean value of membrane potentials, E_m, which are continuously changing (because of the continuous firing of action potentials). In considering this problem, we thought it conceivable that an accurate comparison between E_m and $[Cl]_i$ could be made in in situ hearts of hibernating animals (where the heart rate is extremely slow). However, in searching the literature, we found little if any information regarding Cl distribution in the ventricles of hibernating animals. We decided, therefore, to first examine the Cl distribution in the ventricles of our experimental animal,