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CALCIUM-DEPENDENT K^+ EFFLUX FROM RAT SUBMANDIBULAR GLAND

THE EFFECTS OF TRIFLUOPERAZINE AND QUINIDINE

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The Ca^{2+}-dependent K^+ efflux from rat submandibular gland was studied using a K^+-sensitive electrode. A K^+ efflux was induced by either adrenalin or by using the divalent cation ionophore A23187 plus added Ca^{2+} to bypass the receptor mechanism. Trifluoperazine, which was used to investigate the role of calmodulin, was found to block the adrenalin-induced K^+ efflux but not the A23187/Ca^{2+}-induced K^+ efflux. The adrenalin-induced K^+ efflux was abolished by quinidine and the A23187/Ca^{2+}-induced K^+ efflux was significantly reduced by quinidine. In other experiments, the presence of indomethacin did not inhibit the adrenalin-induced K^+ efflux, and exogenously added arachidonic acid did not induce a K^+ efflux. It is concluded that neither prostaglandin synthesis, nor a cytosolic Ca^{2+}-calmodulin complex is involved in the agonist-induced K^+ efflux from rat submandibular gland. A similarity between the Ca^{2+}-dependent K^+ efflux mechanism of erythrocyte ghosts and submandibular tissue is indicated by their common response to quinidine.

Introduction

Nerve stimulation of salivary gland secretion is accompanied by a substantial loss of glandular K^+ [1]. From this initial observation, subsequent investigations have shown that activation of either cholinoceptors or α-adrenoceptors induces a K^+ efflux from salivary gland acinar cells [2–4]. Both acetylcholine and adrenalin have been proposed to increase cytosolic [Ca^{2+}] [5,6], and it is this increase of cytosolic [Ca^{2+}] which leads to activation of the K^+ efflux from the salivary gland [4]. The mechanism by which an elevated cytosolic [Ca^{2+}] induces membrane permeability changes to K^+ is not understood.

Many Ca^{2+}-dependent processes have been demonstrated to involve a Ca^{2+}-calmodulin complex [7]. It is not known though whether a Ca^{2+}-calmodulin complex is involved in the activation of a K^+ efflux from salivary gland tissue. Both quinine and quinidine have been shown to block the Ca^{2+}-dependent K^+ efflux from erythrocytes [8]. A similarity between the Ca^{2+}-dependent K^+ efflux mechanism of salivary glands and erythrocytes has yet to be described.

A recent report has shown that agonists which are proposed to increase cytosolic [Ca^{2+}] also increase prostaglandin synthesis [9]. However, it is not known if prostaglandin synthesis is required to induce an increase of cytosolic [Ca^{2+}].

In the present report, we have used trifluoperazine [10] to investigate whether a Ca^{2+}-calmodulin complex is involved in the activation of a K^+ efflux from rat submandibular tissue. The effects of quinidine on the Ca^{2+}-dependent K^+ efflux were also examined. Indomethacin and arachid-

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onic acid were used to investigate the role of prostaglandin synthesis in activation of the K^+ efflux. A K^+-sensitive electrode was used to monitor the $[K^+]$ of the incubation medium.

Methods

Female albino rats (200–250 g) were killed by a blow to the head and both submandibular glands were removed. The glands were cut into pieces (approx. 0.5×0.5 mm) with a McIlwain tissue chopper. At all subsequent stages the tissue pieces were maintained in a physiological salt solution at 37°C and gassed continuously with 95% O_2/5% CO_2. The tissue pieces were initially incubated for 2 min, then tranferred to a fresh medium for a further 10 min incubation. The tissue was then transferred to a jacketed water bath, containing 3 ml medium, held at 37°C. Gassing was maintained through a sintered glass support in the bottom of the bath. The $[K^+]$ of the bath was continuously monitored with a K^+-sensitive electrode, constructed in the following manner.

The valinomycin membrane solution was prepared as described by Hill et al. [11]. Two glass tubes (internal diameter = 5 mm) with Corning B7 extended cones at one end were filled with a 2% agar gel containing Na^+ and K^+ at concentrations equivalent to that of the physiological salt solution. Ag/AgCl electrodes were inserted into the agar. The valinomycin membrane mixture was fused to a piece of poly (vinyl chloride) tubing which had been placed over the tip of one electrode. Both electrodes protruded into the lumen of the bath via cone socket adaptors. The electrodes were connected via an Orion model 401 specific ion meter to a Toshin chart recorder and measurements of bath $[K^+]$ were made from these charts.

Agonists or agents used to elicit the K^+ efflux were added to the bath when a stable baseline recording of bath $[K^+]$ was obtained. Results are expressed as the change in bath $[K^+]$ measured from this point. The data in each group of experiments were pooled and the mean and standard error of the mean were calculated for each time point. Student's *t*-test was used to determine the significance between pairs of group means. When quinidine or trifluoperazine were used, they were present in both the wash and bath medium.

The physiological salt solution used for most experiments was Synthetic Interstitial Fluid, (SIF) [12], which contains: 107 mM NaCl/3.48 mM KCl/1.53 mM $CaCl_2$/0.60 mM $MgSO_4$/26.2 mM $NaHCO_3$/1.67 mM NaH_2PO_4/9.64 mM Na gluconate/6.6 mM glucose/7.6 mM sucrose. The Ca^{2+}-free SIF for experiments involving A23187 contained no $CaCl_2$ or $MgSO_4$ and had 10^{-4} M EGTA. Poly(vinyl chloride) for the K^+-selective membranes was a gift from Iplex Plastics, Adelaide. Indomethacin was a gift from Dr. G.C. Scroop and trifluoperazine was given by Smith, Kline and French. Arachidonic acid, valinomycin, quinidine sulphate and adrenalin bitartrate were purchased from Sigma Chemical Company, U.S.A. All other chemicals were reagent grade.

Results

Effects of adrenalin and A23187 on K^+ efflux

Electrode drift has been reported to be a problem in previous studies of net K^+ fluxes in which ion-selective electrodes were used [4], but was not observed in these experiments. When tissue pieces were transferred to the recording bath medium, the bath $[K^+]$, which was initially 3.48 mM, fell by as much as 0.2 mM. Following this period of active uptake (approx. 2–3 min), the bath $[K^+]$ stabilized and under basal conditions remained stable providing that the oxygenation of the medium was maintained at a high rate and tissue pieces circulated freely without sedimenting to the bottom of the bath. The addition of 20 μM adrenalin to the medium produced a rapid rise in bath $[K^+]$ (Fig. 1A). The latency of the response was less than 5 s and the peak in bath $[K^+]$ was reached within 1 min. Subsequent to the peak in bath $[K^+]$ there was an initial rapid fall in bath $[K^+]$, which lasted 2–3 min, followed by a prolonged phase during which bath $[K^+]$ gradually declined. When the divalent cation ionophore A23187 was added to a Ca^{2+}-free medium, the bath $[K^+]$ rose by up to 0.3 mM (Fig. 1B). The subsequent addition of 1.63 mM $CaCl_2$ produced a rapid rise in the bath $[K^+]$. The time course and magnitude of this Ca^{2+}-induced K^+ efflux was the same as that induced by adrenalin in a normal SIF medium.

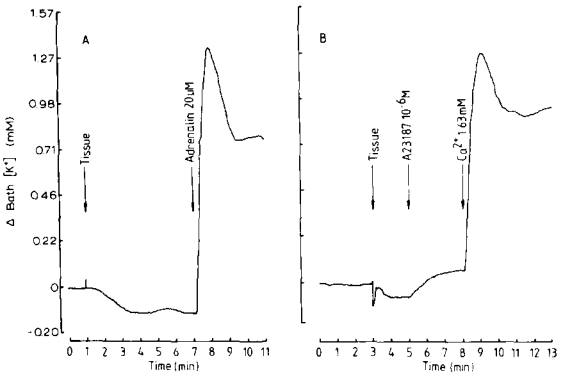

Fig. 1. Time course and characteristics of K^+ efflux from rat submandibular gland. (A) Adrenalin-induced K^+ efflux. In this experiment and those to be described subsequently in which adrenalin was the agonist, the medium used throughout the experiment was normal SIF. Tissue was transferred to the recording bath at $t = 1$ min. Following the stabilization in bath $[K^+]$, 20 μM adrenalin was added at $t = 7$ min. (B) For experiments involving the use of A23187 plus added Ca^{2+} to induce a K^+ efflux, the medium used throughout was a Ca^{2+}-free SIF which contained 10^{-4} M EGTA but not Ca^{2+} or Mg^{2+}. Tissue was transferred to the recording bath at $t = 3$ min. 10^{-6} M A23187 was added at $t = 5$ min and 1.63 mM Ca^{2+} added at $t = 8$ min. Both Fig. 1A and B are reproductions of actual chart recordings.

The effects of trifluoperazine on K^+ efflux

Trifluoperazine at the concentrations used, was found to alter the characteristics of the K^+-sensitive electrode. For example, at 10^{-4} M trifluoperazine, the electrode calibration was reduced by up to 2 mV/decade change $[K^+]$. Thus, for each experiment, the electrode was calibrated at the end of the procedure.

At both 10^{-6} M and 10^{-5} M, trifluoperazine significantly reduced the peak bath $[K^+]$ in response to 20 μM adrenalin ($P < 0.05$) while 10^{-4} M trifluoperazine abolished the adrenalin response (Fig. 2). When a K^+ efflux was induced by A23187 and Ca^{2+}, 10^{-4} M trifluoperazine significantly enhanced the resultant K^+ efflux for the first 2 min following the addition of Ca^{2+} ($P < 0.05$, Fig. 3). As the net efflux measured in these experiments is the difference between passive efflux and active influx, the experiments were repeated in the presence of 10^{-4} M ouabain to determine if inhibition of the $Na^+ + K^+$ pump by trifluoperazine contributed to the enhanced K^+. Following the addition of Ca^{2+}, the presence of ouabain did not prevent trifluoperazine from significantly enhancing the Ca^{2+}-induced K^+ efflux ($P < 0.05$, Fig. 3).

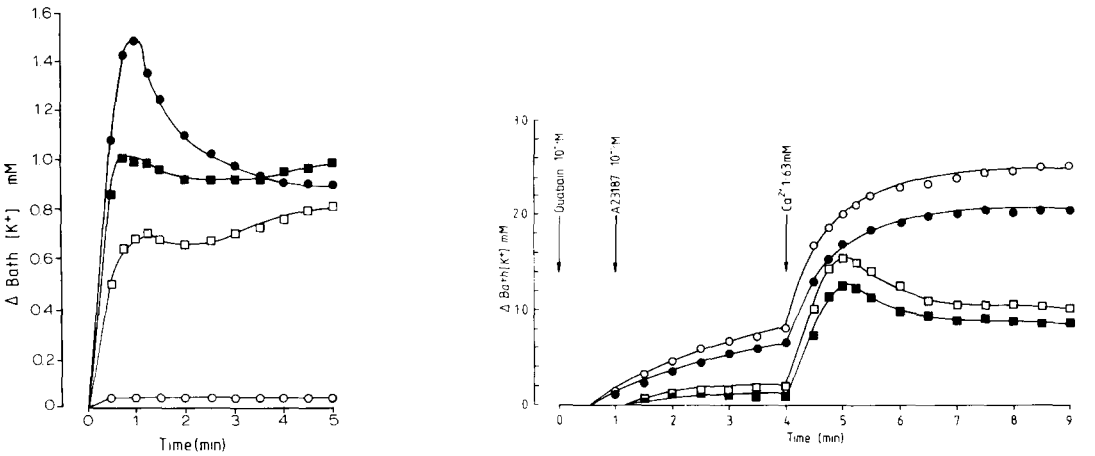

Fig. 2. Trifluoperazine and adrenalin-induced K^+ efflux. Tissue pieces were pre-incubated in the presence of trifluoperazine for 10 min, at concentrations of 0 (●); 10^{-6} M (■); 10^{-5} M (□) or 10^{-4} M (○). The recording bath medium contained the appropriate concentration of trifluoperazine. 20 μM adrenalin was added at $t = 0$. For each group of experiments, $n = 6$. Standard error of the mean for any time point did not exceed $\pm 12\%$ of the mean.

Fig. 3. Trifluoperazine and Ca^{2+}-induced K^+ efflux. The results from two sets of experiments are shown. For one group (●, ○) 10^{-4} M ouabain was added 1 min prior to the addition of A23187. Ouabain was not used for the other group of experiments (■, □). Trifluoperazine was present throughout the experiments (○, □) while the other two graphs (●, ■) are the respective controls. Each curve is the mean of five separate experiments. For the group of experiments (□, ■) the time points were significantly different for the first 2 min after the addition of Ca^{2+} ($P < 0.05$). For the other group (○, ●) all time points following the addition of Ca^{2+} were significantly different ($P < 0.05$). The standard error of the mean did not exceed $\pm 10\%$ of the mean for any time point.

The effects of quinidine on K^+ efflux

The adrenalin-induced K^+ efflux was abolished by the presence of 10^{-3} M quinidine, while both 10^{-4} and 10^{-5} M quinidine significantly reduced the peak bath $[K^+]$ in response to 20 μM adrenalin ($P < 0.05$; Fig. 4A). When Ca^{2+} with A23187 was used to induce a K^+ efflux, 10^{-3} M quinidine significantly reduced the rise in bath $[K^+]$ for the first 1.25 min after the addition of Ca^{2+} ($P < 0.05$; Fig. 4B). Also, whereas in the control experiments a characteristic uptake of K^+ occurred following the peak in bath $[K^+]$, no such peak or uptake was observed when quinidine was present. Rather, the bath $[K^+]$ continued to rise gradually reaching a plateau which was significantly elevated above the level for the control curve ($P < 0.05$; Fig. 4B). To exclude any possible effects of quinidine on the $Na^+ + K^+$ pump, the experiments were repeated in the presence of 10^{-4} M ouabain. The Ca^{2+}-induced K^+ efflux, in the combined presence of quinidine and ouabain was still reduced for the first 2 min following the addition of Ca^{2+} (Fig. 5A). However, the bath $[K^+]$, prior to the addition of Ca^{2+}, was lower when both quinidine and ouabain were present compared to ouabain alone (Fig. 5A). Therefore, in order to determine if quinidine had effectively reduced the K^+ efflux, the rate of change of bath $[K^+]$ was calculated (Fig. 5B). The rate of change of bath $[K^+]$ was significantly reduced for the first 0.5 min following Ca^{2+} addition in the presence of quinidine ($P < 0.05$).

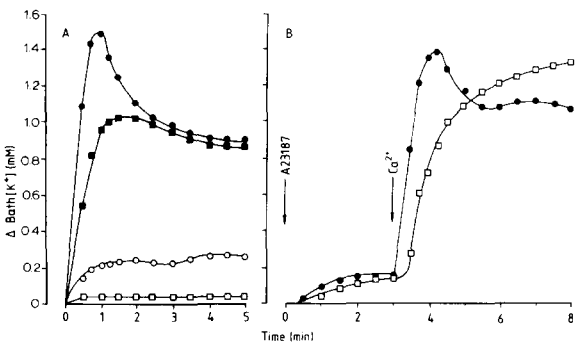

Fig. 4. The effects of quinidine on K^+ efflux from submandibular tissue. (A) Adrenalin-induced K^+ efflux and quinidine. The preincubation and bath medium contained quinidine at concentrations of 0 (●); 10^{-5} M (■); 10^{-4} M (○) or 10^{-3} (□). The period of pre-incubation in the presence of quinidine was 10 min for all experiments. 20 μM adrenalin was added at $t = 0$. Each time point is the mean of at least five separate experiments. Standard errors of the mean did not exceed $\pm 10\%$ of the mean for any time point. (B) Quinidine and Ca^{2+}-induced K^+ efflux. The pre-incubation and bath medium contained quinidine at concentrations of 0 (●) or 10^{-3} M (□). Each time point is the mean of five separate experiments and the standard error of the mean for all time points was less than $\pm 10\%$.

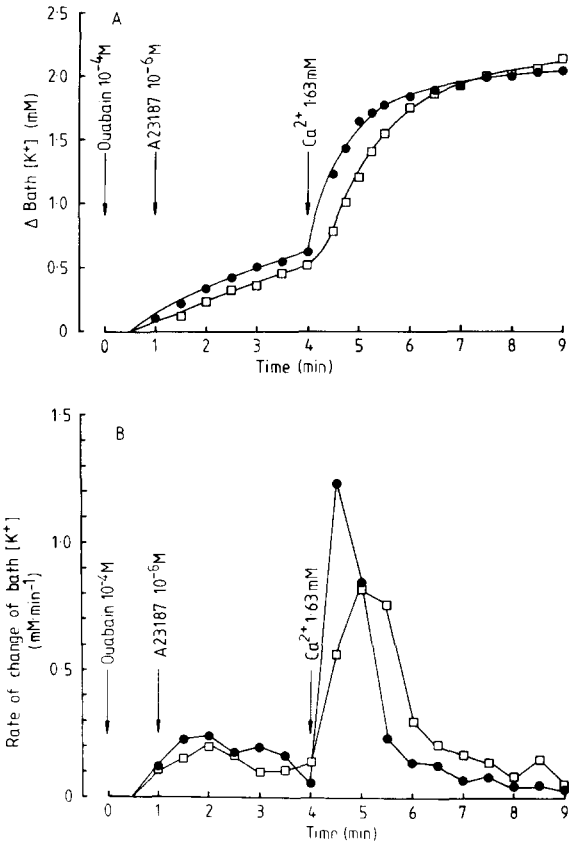

Fig. 5. The effects of quinidine on K^+ efflux from submandibular tissue. (A) The experimental protocol is the same as that described in Fig. 4B except that 10^{-4} M ouabain was added 1 min prior to the addition of A23187. Control (●), 10^{-3} M quinidine (□). Standard errors of the mean did not exceed $\pm 10\%$ mean. (B) Rate of change of bath $[K^+]$ was calculated for each individual experiment and the results pooled. The rate of change of bath $[K^+]$ was significantly reduced for the first 0.5 min following the addition of Ca^{2+}. Standard errors of the mean did not exceed $\pm 10\%$ for any time point.

Prostaglandin synthesis and K^+ efflux

When 10^{-4} M arachidonic acid was added to

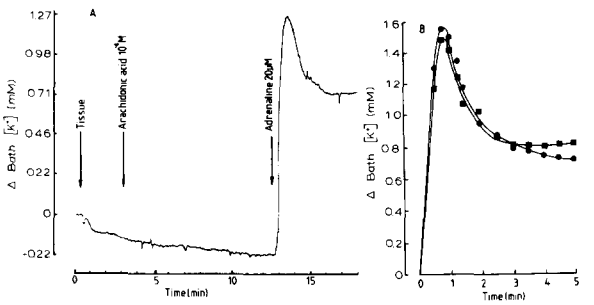

Fig. 6. Prostaglandin synthesis and K^+ efflux. (A) Shown is a chart recording from one of three separate experiments. Exogenously added arachidonic acid was given at $t = 3$ min. Tissue integrity is demonstrated by a normal response to adrenalin at $t = 12.5$ min. (B) Tissue was incubated in the presence of $5 \cdot 10^{-5}$ M indomethacin (■) or its vehicle (●) for 30 min prior to transfer to the recording bath medium which also contained indomethacin or its vehicle. Each time point is the mean of three separate experiments. Standard error of the mean for any time point did not exceed ±15% of the mean.

the bath medium, the bath $[K^+]$ remained at basal levels (Fig. 6A). No response was observed even after arachidonic acid had been present for 10 min. Tissue integrity was demonstrated by a normal response to adrenalin. A 30 min preincubation of tissue pieces in the presence of $5 \cdot 10^{-5}$ M indomethacin before transfer of the tissue to the bath medium which also contained indomethacin did not prevent a normal adrenalin-induced K^+ efflux (Fig. 6B).

Discussion

The reproduction of chart recordings in Fig. 1A and B demonstrate the significant improvement in resolution of net K^+ fluxes using a K^+-sensitive electrode, compared with an earlier study [4]. The sensitivity of the technique is reflected by the demonstration of an A23187-induced K^+ efflux in the absence of external Ca^{2+}. This response was not abolished by raising the EGTA concentration to 2 mM or by the presence of a combined autonomic blockage (Kurtzer, R. and Roberts, M.L., unpublished data). Since the effective $[Ca^{2+}]$ of a Ca^{2+}-free medium containing 10^{-4} M EGTA is computed to be of the order to 10^{-9} M [13], it would appear that A23187 has released EGTA-inaccessible Ca^{2+} from internal stores to the cytosol, thereby activating a K^+ efflux.

Trifluoperazine, a calmodulin antagonist [10] was used in the present study to investigate the possible involvement of calmodulin in activation of the Ca^{2+}-dependent K^+ efflux from rat submandibular gland. The finding that trifluoperazine blocked the adrenalin-induced K^+ efflux, but not the A23187/Ca^{2+}-induced K^+ efflux reinforces a growing body of evidence which cautions the interpretation of trifluoperazine's action when using whole tissue preparation [14,15]. As trifluoperazine did not inhibit the K^+ efflux activated by Ca^{2+} plus A23187 it is unlikely that a cytosolic calmodulin is involved in activation of the K^+ efflux. Trifluoperazine potentiation of the K^+ efflux induced by Ca^{2+} plus A23187 may be explained by the phenothiazine causing a reduction of Ca^{2+}-ATPase activity leading to a persistence of elevated cytosolic $[Ca^{2+}]$. Trifluoperazine could reduce Ca^{2+}-ATPase activity by a direct effect on the Ca^{2+}-ATPase [16], reduction of cellular ATP levels [17], or expansion of the lipid bilayer [18] due to trifluoperazine's lipophilic nature [19].

Although the inhibitory effect of quinidine on the K^+ efflux in the present study was not as pronounced as for the Ca^{2+}-dependent K^+ efflux in the erythrocyte [8], the data presented in Fig. 5A and B indicates that quinidine has slowed the efflux of K^+. These results would then suggest that the K^+ permeability mechanism of the submandibular tissue resembles, to some extent, that described for erythrocyte ghosts [8]. It should also be noted that quinidine has an adrenoceptor-blocking effect which was also observed for the adrenoceptors of mouse parotid gland [20]. An explanation as to why there is no reuptake of K^+ observed in the presence of quinidine (Fig. 4B) is not readily apparent.

At the time of preparation of the present paper, there appeared a report showing that the agonist-induced K^+ efflux in rat parotid tissue is not associated with prostaglandin synthesis [21]. Results obtained in the present investigation confirmed this initial report and further extend the finding to include rat submandibular tissue. A possible criticism which could be levelled at experiments involving the addition of arachidonic acid to organ baths arises from the apparent ease with which arachidonic acid can be oxidized. We

found that incubating arachidonic acid for 10 min in a continuously-gassed medium reduced the arachidonic acid concentration from $1.2 \cdot 10^{-4}$ to $0.9 \cdot 10^{-4}$ M (Kurtzer, R.J. and Roberts, M.L., unpublished data).

In summary, it has been demonstrated that the use of a K^+-sensitive electrode to monitor net K^+ flux from rat submandibular tissue incubated in vitro is a simple but sensitive technique. The results presented in this report indicate that it is unlikely that a cytosolic Ca^{2+}-calmodulin complex mediates the agonist-induced K^+ efflux from rat submandibular tissue. Further, prostaglandin synthesis has been excluded from playing a major role in the agonist-induced K^+ efflux. A similarity of the Ca^{2+}-dependent K^+ efflux mechanism of erythrocyte ghosts and rat submandibular tissue is indicated by their common response to quinidine.

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