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Effect of Chronic Reserpine Administration on K^+ and Amylase Release from the Rat Parotid Gland

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Summary

The release of K^+ and of amylase were compared in parotid gland slices from control and reserpine-treated rats during incubation in an enriched, oxygenated medium. After 10 min of incubation in the presence of 2×10^{-5} M epinephrine, net K^+ release was 17% in control slices and 32% in those from the treated animals. When 1 mM ouabain was added to the incubation medium in the presence of the hormone, K^+ release was similar in the two types of slices and reached 50%. Omission of Ca^{++} from the incubation medium reduced the epinephrine-induced net release in control slices to 9%, but not in those of reserpine-treated rats, which still showed a 28% release. The divalent cation ionophore A 23187 also induced a significantly greater K^+ release in the slices of the treated animals. In the absence of ouabain, the 8-bromo derivative of cyclic GMP inhibited basal K^+ release in control slices and caused a small, but significant K^+ uptake in the slices of the treated animals. Amylase release after 30 min of incubation in the presence of either 2×10^{-5} M epinephrine or 10^{-5} M isoproterenol was 50% in control slices and 17% in slices from reserpine-treated rats. Amylase release was also reduced in the slices of the treated animals in the presence of the dibutyryl derivative of cyclic AMP. Release of this enzyme was not affected by the presence of ouabain or by the omission of Ca^{++} from the incubation medium, but was significantly reduced in the slices of both control and reserpine-treated rats when the Na^+ concentration of the incubation medium was reduced to 21 mM. Amylase release was reduced in control slices to the levels observed in slices of reserpine-treated rats when the purines inosine and adenine were omitted from this medium. The chronic administration of reserpine, thus, results in a significantly increased release of K^+ and in a significantly decreased release of amylase from rat parotid gland slices. These results demonstrate that chronic reserpine administration inpairs the *in vitro* responses of both α and β adrenergic receptors in the rat parotid gland.

Speculation

Prolonged reserpine administration impairs the stimulus-secretion coupling mechanism in the parotid glands, leading to an altered secretion of ions and of macromolecules. Because the reserpine-treated rat has been proposed as an animal model for cystic fibrosis, these findings suggest that the secretory abnormality observed in this disease is also related to an abnormal stimulus-secretion coupling mechanism during the secretory response to physiologic stimulants.

It has been clearly demonstrated that slices of both the parotid (2, 3, 5, 6, 13, 15, 16) and the submaxillary glands (9, 11) incubated in vitro release K^+ ions upon stimulation of cholinergic and of α-adrenergic receptors. Parotid slices also release amylase after stimulation of β-adrenergic receptors (1, 4, 14, 15). The release of K^+ has been shown to be dependent on the presence of Ca^{++} in the incubation medium in both the parotid and submaxillary slice systems (9, 11, 15) and to be the result of two opposing and almost simultaneous mechanisms, a passive K^+ efflux and an active K^+ reuptake that depends on the activation of a ouabain-sensitive Na^+, K^+, ATPase. The release of amylase that occurs after β-receptor stimulation in the parotid gland presumably depends on the stimulation of cyclic AMP synthesis (1, 4, 6, 14, 15), that, in turn, is thought to release Ca^{++} from intracellular storage sites. The released Ca^{++} may act as the final mediator in the discharge of zymogen granules (14).

Other evidence has indicated that the chronic administration of reserpine to rats results in an increased net release of K^+ from submaxillary slices after stimulation of cholinergic or α-adrenergic receptors, most likely as a result of decreased rates of K^+ reuptake (12). The effects of pretreatment with reserpine on the response of β-adrenergic receptors *in vitro* has not been assessed. *In vivo*, the drug treatment was found to alter the secretory response to isoproterenol in the submaxillary gland (12). The parotid slice system has been used in this study, therefore, to investigate the effects of chronic reserpine administration on the responses of α and β adrenergic receptors *in vitro*. Because Ca^{++} and the cyclic nucleotides are involved in these responses, the authors have also measured the release of K^+ and of amylase in the presence of the divalent cation ionophore A 23187 and of exogenous derivatives of cyclic AMP and cyclic GMP.

METHODS

Adult, male albino rats of the Sprague-Dawley strain were treated for 7 days with a 0.5 mg/Kg daily dose of reserpine, as previously described (10) and housed in the same quarters with untreated controls. All animals were provided with water *ad libitum* and with a standard pelleted diet. Twenty-four hr after the 7th reserpine injection, the rats were anesthetized with sodium pentobarbital (8 mg/100 g body wt), killed by air infusion into the thorax, and the bloodless parotid glands were removed, trimmed of lymph nodes and fat, and rapidly placed in a beaker containing 20–30 ml of oxygenated Krebs-Ringer bicarbonate solution. The glands from 3–4 rats were then cut with a sharp scalpel blade in a large drop of medium over a parafilm sheet into slices of approximately 1 mm^3, pooled, and divided into 3 or 4 nitrocellulose tubes containing 2 ml of fresh medium. Each tube contained the equivalent of two glands (250–300 mg). After a 15-min period, the preincubation medium was carefully decanted, the slices were washed with fresh medium and placed in 2 ml of new medium for the final incubation. Stimulants were added to the slice systems at the start of the final incubation in 50 μl volumes and 25–50 μl samples of the medium were removed at timed intervals and placed in small tubes kept in ice. Incubation of the slices was carried out in a constant temperature water shaker bath kept at 37°C and was prolonged for 30 or more min with continuous O_2-CO_2 bubbling and gentle shaking. At the end of this period, the slices were homogenized in the remaining medium with a Polytron homogenizer. The K^+ and amylase concentrations in the aliquots of medium removed in the course of the experiments and in the slice homogenate were determined in duplicate, respectively, in an Instrumentation Laboratories flame photometer

and by the Harleco method (Harleco Co., Gibbstown, NJ, amylase reagent set #64191). The latter is a colorimetric method which compares the difference in absorbance of a starch-iodine complex in the sample versus that of a blank. A unit of amylase is defined as the amount of enzyme that will hydrolyze 10 mg of starch in 30 min. Using the values thus obtained, the release of K^+ and of amylase were expressed as a percent of the respective amounts present in the slices, by using formulae previously described (1, 2, 9, 10, 11). The same procedures were carried out in similarly prepared slices from untreated controls, so that paired observations were made in each of the experiments performed.

Both the preincubation and the final incubation of the slices were carried out in Krebs-Ringer bicarbonate medium that had been warmed to 37° C and gassed with a 95% O_2-5% CO_2 mixture for at least 30 min before the start of the experiment. The composition of the incubation solution was as previously described (10). In some experiments, ionic substitutions in the medium were made and the slices were either preincubated or incubated in the substituted medium. These substitutions included: 1) the removal of K^+ or of Ca^{++}; 2) the lowering of the Na^+ to 21 mM. In this case, Na^+ was substituted by an equal amount of lithium; 3) the removal of the purines inosine and adenine. In other experiments, the final incubation was carried out in Krebs-Ringer bicarbonate medium containing 1 mM ouabain.

Secretion of K^+ and of amylase from the slices was assessed in the presence of the following agents: 1) epinephrine bitartrate, and d, l-isoproterenol sulfate; 2) the 8-bromo derivate of cyclic guanosine monophosphate and the dibutyril derivative of cyclic adenosine monophosphate. These agents and ouabain were purchased from Sigma Chemical Company (St. Louis, MO); 3) the divalent cation ionophore A 23187, which was a generous gift from Eli Lilly Company, Indianapolis, IN; 4) phentolamine or propranolol were used in experiments designed to test the effect of receptor blocking agents. The first was kindly provided by Ciba Pharmaceutical Company, Summitt, NJ and the latter by Ayerst Laboratories, Montreal, Canada. The dosages of the various stimulants and the incubation conditions of each experiment are described in the results section. Each type of experiment was repeated at least four times and mean values for amylase and K^+ release were obtained and statistically compared by using Student's *t* test.

RESULTS

BASAL RELEASE OF K^+ AND OF AMYLASE

When the parotid slices from control and reserpinized rats were incubated in the absence of secretagogue, the extent of amylase release after 30 min of incubation was 3.7 ± 0.2% and 4.4 ± 0.9%, respectively. Potassium release after 10 min of incubation, on the other hand, was 10.6 ± 2.5% in control slices and 8.7 ± 1.2% in the slices of the treated animals. No significant difference in the basal (unstimulated) release of K^+ or of enzyme was, therefore, found between the two types of slice preparation.

EFFECTS OF EPINEPHRINE AND OF ISOPROTERENOL

Stimulation of the parotid slice systems with 2×10^{-5} M epinephrine resulted in a significantly larger net K^+ release ($P < 0.005$) and in a significantly smaller amylase release ($P < 0.005$) from the slices of reserpine-treated rats. These findings are illustrated in Figure 1, which show that the parotid slices from untreated controls had a net K^+ release of 17% after 10 min of incubation, whereas those of reserpine-treated rats had a 32% net K^+ release after the same incubation time. Amylase release from control slices was 54% after 30 min of incubation, whereas that from the slices of the treated animals was only 18% at this time.

Figure 2 illustrates the findings obtained when the slice systems of both groups of animals were stimulated with 10^{-5} M isoproterenol. As previously shown in both the parotid (1, 3) and submaxillary (11) slice systems of normal rats, this secretagogue fails to induce a net K^+ release after 10 min of incubation. In the present experiments, the net K^+ release ascertained in control slices was 4.5%, a value not significantly different than that observed during basal (unstimulated) conditions. In the slices from reserpine-treated rats, net K^+ release after isoproterenol was significantly smaller (−0.6%). As in the case of epinephrine, amylase release from the slices of the treated animals was significantly lower ($P < 0.005$) than in control slices after 30 min of incubation and reached 17%, as opposed to 50% in control slices.

EFFECT OF ANTAGONISTS

On the basis of the findings obtained in the experiments with epinephrine, the effect of specific α and β adrenergic receptor antagonists was studied in additional experiments in which the

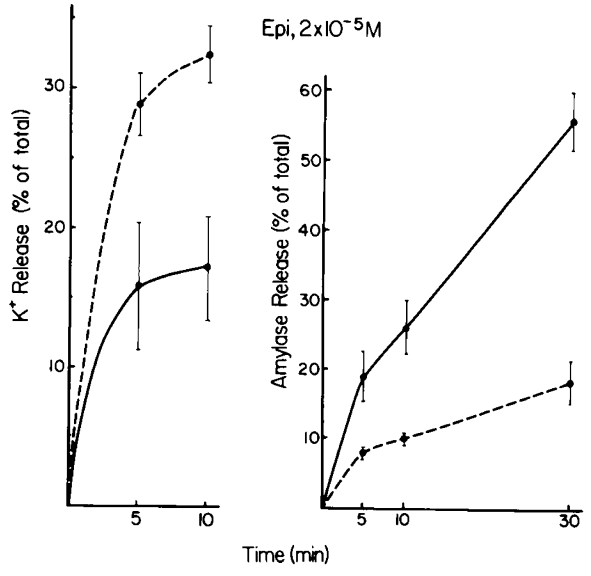

Fig. 1. Release of K^+ and of amylase from parotid slices of control (*dark symbols*) and reserpine-treated rats (*light symbols*) after stimulation with epinephrine. Note that in this and the subsequent figures, the scale for percent K^+ release (*vertical axis*) is twice of that for amylase release and that the scale for time (*horizontal axis*) has been expanded for the K^+ release curves. *Vertical bars* represent standard errors. Number of experiments: control = 6; reserpine = 6.

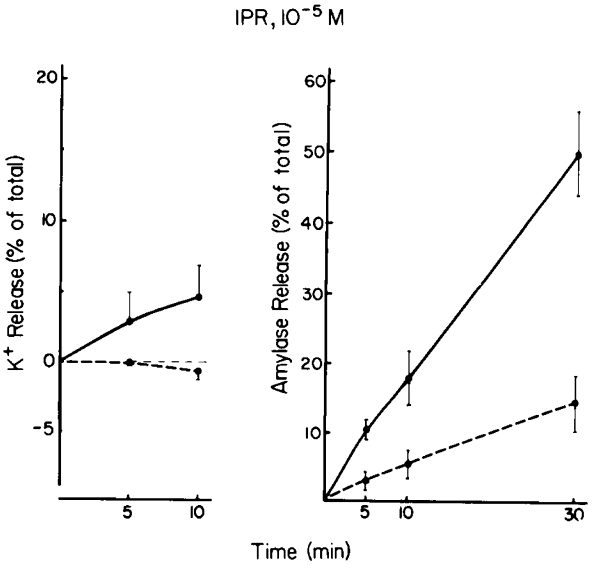

Fig. 2. Release of K^+ and of amylase from parotid slices of control and reserpine-treated rats after stimulation with isoproterenol. Symbols as in Figure 1. Number of experiments: control = 4; reserpine = 4.

antagonist was added to the incubation medium 10 min before the hormone. Figure 3 shows the effect of the specific α-receptor antagonist phentolamine on K^+ and amylase release from the parotid slice systems of control and reserpine-treated rats. Blockade of this receptor resulted in an inhibition of K^+ release in both types of slices, so that a net K^+ uptake into the slices was observed. This uptake, however, was significantly greater in the slices of the reserpine-treated animals ($P < 0.05$). Phentolamine, on the other hand, did not significantly affect amylase release in the slices of control and reserpine-treated animals. The extent of amylase release observed in the presence of this antagonist in control slices (53%) or in the slices of reserpine-treated animals (21%) was not different from that seen after epinephrine alone as shown in Figure 1. In both cases, however, the release of amylase was significantly lower in the slices of reserpine-treated animals.

When the specific β-receptor antagonist propranolol was added to the incubation medium 10 min before epinephrine, the results were as illustrated in Figure 4. There was a reduction in the net K^+ release from both control slices and those of reserpine-treated animals, which may have resulted from a local anesthetic effect of propranolol (2). Amylase release was also reduced in both types of slices to 50% of the respective values observed after epinephrine alone. Enzyme release was still significantly lower in the slices from reserpine treated animals in the presence of propranolol (Fig. 4).

EFFECTS OF OUABAIN AND OF LOW EXTERNAL NA^+

The effects of ouabain and of a reduced external Na^+ concentration of stimulation-induced K^+ and amylase release were assessed in the parotid slice systems of control and reserpine-treated rats. The results are summarized in Table 1. Addition of ouabain to the final incubation medium resulted in a large K^+ release from both types of slices after epinephrine stimulation. No significant difference between the respective values was noted. Amylase release after this secretagogue in the presence of the glycoside was not significantly different than that obtained with epinephrine alone in either the slices of control or reserpine-treated rats. As in the case of the hormone alone, however, the release of amylase was significantly lower ($P < 0.005$) in the slices of the treated animals (Table 1). A small K^+ efflux was observed in the presence of ouabain when isoproterenol was used as the secretagogue (Table 1), and it was significantly higher ($P < 0.05$) in the slices of the treated animals. The presence of ouabain in the incubation medium did not affect the release of amylase induced by isoproterenol in either control slices or those from reserpine-treated rats

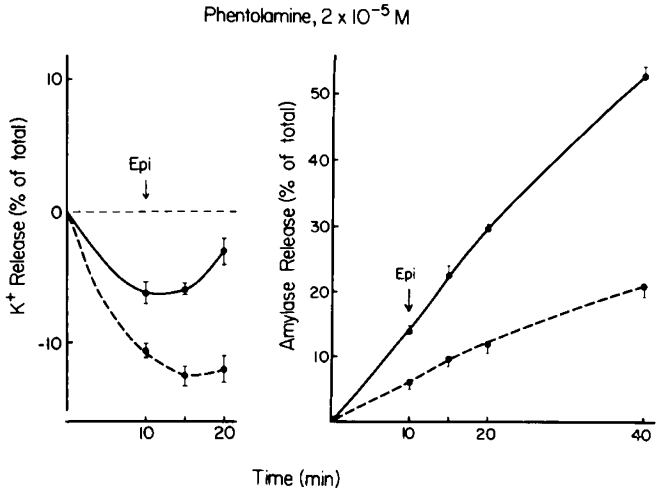

Fig. 3. Release of K^+ and of amylase from parotid slices of control and reserpine-treated rats when the α-receptor antagonist phentolamine (2×10^{-5} M) was added to the incubation medium at zero time, 10 min before epinephrine (2×10^{-5} M). Number of experiments: control = 6; reserpine = 6.

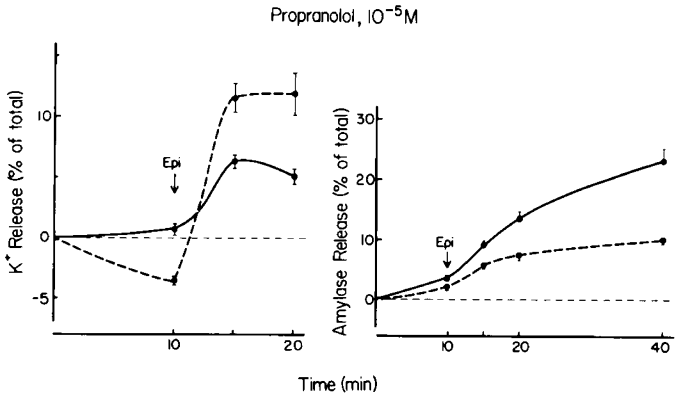

Fig. 4. Release of K^+ and of amylase from parotid slices of control and reserpine-treated rats when the β-receptor antagonist propranolol (10^{-5} M) was added to the incubation medium at zero time, 10 min before epinephrine (2×10^{-5} M). Number of experiments: control = 4; reserpine = 4.

(Table 1), although the percent of amylase released was still significantly smaller ($P < 0.005$) in the slice systems from reserpine-treated rats.

Reducing the Na^+ concentration of the incubation medium to 21 mM resulted in an enhanced K^+ efflux from the slices of control, but not of reserpine-treated rats, after epinephrine stimulation (Table 1). It also caused a 50% reduction in the amount of amylase released by this secretagogue in both types of slices, with the release from slices of reserpine-treated rats still being about one-half of that elicited from control slices. When isoproterenol was used as the stimulant, the extent of K^+ efflux observed in the low Na^+ incubation medium was not as high as that seen with epinephrine (Table 1). In this case, however, the K^+ efflux was lower in the slices from the treated rats. The reduction in external Na^+ also reduced in a significant manner the amount of amylase released by isoproterenol stimulation in both control slices and those of reserpine-treated rats (Table 1). The latter released a significantly smaller amount of amylase than control slices under these incubation conditions ($P < 0.005$).

EFFECTS OF CA^{++} REMOVAL

Experiments were performed to investigate the Ca^{++} dependency of the stimulation-induced K^+ and amylase release in the slices of control and reserpine-treated rats. The results are summarized in Table 2. In the first group of experiments, Ca^{++} was omitted from the incubation solution and ion and enzyme release were measured in the presence of epinephrine and isoproterenol. In the absence of external Ca^{++}, epinephrine-induced K^+ release was decreased in control slices, but not in the slices from reserpine treated rats (Table 2). By contrast, the release of amylase induced by epinephrine was not reduced in the absence of external Ca^{++} in either control slices or those from reserpine-treated rats (Table 2). In the Ca^{++}-free medium, isoproterenol caused a significant K^+ uptake in both types of slices, which was significantly greater ($P < 0.05$) in the slices of reserpine treated rats (Table 2). As in the case of epinephrine, Ca^{++} removal did not modify the amylase released after stimulation with isoproterenol (Table 2). As previously noted, in every type of experiment described to this point, amylase release under these conditions was significantly smaller in the slices from reserpine-treated rats.

The second group of experiments performed to test the Ca^{++} dependency of the release mechanisms for K^+ and for amylase involved the addition of lanthanum to the incubation medium 10 min before the secretagogues. The results are also shown in Table 2. This procedure, like the removal of Ca^{++} from the medium, reduced the epinephrine-induced K^+ release in control slices, but not in the slices of reserpine-treated rats. It also reduced slightly the epinephrine-induced amylase release from the former (Table

Table 1. *Effects of ouabain and reduced external Na^+ on K^+ and amylase release*

Incubation conditions	Secretagogue	Type of gland	K^+ release[1] (% of total)	Amylase release[2] (% of total)
KRB + 1 mM Ouabain	Epinephrine	Control	52.6 ± 3.4	45.9 ± 1.2
		Reserpine	47.5 ± 3.1	16.4 ± 2.5
	Isoproterenol	Control	6.1 ± 1.3	43.1 ± 4.8
		Reserpine	16.4 ± 1.5	22.3 ± 3.1
KRB − 21 mM Na^{+}[3]	Epinephrine	Control	44.8 ± 1.9	26.8 ± 5.2
		Reserpine	29.3 ± 3.2	9.3 ± 1.5
	Isoproterenol	Control	15.8 ± 0.7	34.4 ± 1.3
		Reserpine	7.4 ± 1.2	12.7 ± 0.9

[1] K^+ release after 10 min of incubation.
[2] Amylase release after 30 min of incubation.
[3] Na^+ replaced by Li.

Table 2. *Effect of calcium removal on K^+ and amylase release*

Incubation conditions	Secretagogue	Type of gland	K^+ release[1] (% of total)	Amylase release[2] (% of total)
KRB − Ca^{++}	Epinephrine	Control	9.6 ± 1.0	39.9 ± 3.8
		Reserpine	28.2 ± 2.7	19.0 ± 4.3
	Isoproterenol	Control	−6.0 ± 1.4	41.6 ± 6.5
		Reserpine	−16.6 ± 2.8	12.6 ± 1.4
KRB + Lanthanum 10^{-3} M[3]	Epinephrine	Control	10.6 ± 1.2	35.2 ± 4.8
		Reserpine	25.6 ± 3.5	19.3 ± 0.4
	Isoproterenol	Control	−1.2 ± 1.8	36.0 ± 0.4
		Reserpine	−0.7 ± 3.4	17.2 ± 1.8

[1] K^+ release after 10 min of incubation.
[2] Amylase release after 30 min of incubation.
[3] Lanthanum added 10 min before the secretagogue.

2). Similar results were obtained for amylase release when isoproterenol was used as the stimulant (Table 2). As when Ca^{++} was removed from the incubation medium, stimulation of the slices with isoproterenol in the presence of lanthanum resulted in K^+ uptake (Table 2).

EFFECT OF A DIVALENT CATION IONOPHORE AND OF EXOGENOUS DERIVATIVES OF CYCLIC NUCLEOTIDES

The release of K^+ and amylase from the parotid slice systems of control and reserpine-treated rats were assessed in the presence of the divalent cation ionophore A 23187, the 8-bromo derivative of cyclic GMP, and the dibutyryl derivative of cyclic AMP. The effect of these three agents on K^+ release are illustrated in Figure 5. The ionophore is the only one that induced K^+ release, as illustrated in Figure 5. This effect was significantly greater in the slices of reserpine-treated rats ($P < 0.05$). The cyclic GMP derivative, on the other hand, inhibited basal K^+ release in control slices and, in the slices of the treated rats, caused a small, but significant K^+ reuptake. The effect of cyclic AMP on K^+ release, on the other hand, was not significantly different from basal (unstimulated) release in the slices of control and treated rats.

Figure 6 illustrates the effects of these substances on amylase release. In control slices, neither the ionophore A 23187 nor cyclic GMP caused release of amylase. Dibutyryl cyclic AMP caused a small extent of amylase release, which was approximately one-third of the release caused by epinephrine or by isoproterenol. In the slices from reserpine-treated rats, the ionophore induced amylase release to the same extent as the two adrenergic secretagogues and the cyclic AMP derivative induced a 10% release of enzyme. As in the case of control slices, the cyclic GMP derivative did not induce amylase release in these slices.

EFFECT OF SUBSTRATE REMOVAL

Experiments were performed, to evaluate the effects of substrate removal on the K^+ and amylase release elicited by the various stimulants from the parotid slices systems of control and reserpine-treated rats. The results are summarized in Table 3. In these experiments, both the preincubation and the final incubation were carried out in Krebs-Ringer bicarbonate medium from which the purines inosine and adenine were omitted. In general, the purines did not affect the K^+ release induced by the agents listed, although it tended to be generally higher than in complete KRB medium, particularly in control slices. The inhibition of basal release caused by the 8-bromo derivative of cyclic GMP (see Figure 5) was not apparent in the absence of the purines (Table 3) and K^+ release was increased in the slices from reserpine-treated rats, but not in control slices, when the dibutyryl derivative of cyclic AMP was added in the medium containing no purines (Table 3). Omission of these substrates from the incubation medium markedly impaired the amylase release induced by epinephrine or by isoproterenol in control slices and, to a lesser extent, in slices from reserpine-treated rats (Table 3).

DISCUSSION

The results of these experiments demonstrate that the chronic administration of reserpine impairs the *in vitro* response of both α and β adrenergic receptors in the rat parotid gland, and results in a significantly greater net release of K^+ ions and in a significantly smaller amylase release than those observed in tissue slices from untreated rats.

The increased net release of K^+ observed in parotid gland slices of reserpine-treated rats in the presence of epinephrine does not

seem to be due to an increased passive efflux of K^+. Thus, when K^+ uptake was inhibited by the addition of ouabain, the extent of K^+ release was similar to that observed in control slices. It could result, on the other hand, from a reduced reuptake of K^+ under normal conditions of incubation (*i.e.*, in the absence of the glycoside). Because the simultaneous activation of K^+ uptake and of enzyme release in parotid slices seems to require a critical supply of energy (16), K^+ uptake can be reduced in the slices of reserpine-treated animals if this supply of energy is compromised to the point that it cannot fulfill the demands of both mechanisms when both α and β receptors are stimulated simultaneously.

In control parotid slices, activation of the Na^+-K^+ pump appears to require both an influx of Na^+ and the generation of cyclic GMP. Thus, K^+ release was enhanced in these slices when the Na^+ concentration of the medium was reduced to 21 mM and the exogenous derivative of cyclic GMP inhibited K^+ release. An increase in the internal Na^+ concentration of salivary cells had been found to be an effective means of stimulating the pump, whether produced by prolonged exposure to ouabain or by cooling (8). The level of cyclic GMP in the parotid gland, on the other hand, rises rapidly upon stimulation of α-adrenergic receptors (6) or upon exposure to the divalent cation ionophore A 23187 (4, 6), most likely as a result of the activation of guanylate cyclase by a stimulation-induced influx of Ca^{++}. The results of the present experiments confirm the Na^+ requirement for activation of the Na^+-K^+ pump and also indicate that cyclic GMP has a role in this activation. They further suggest that these mechanisms are impaired in the parotid gland of the reserpine-treated animals. Thus, a reduction in the Na^+ concentration of the incubation medium hardly affected the epinephrine-induced K^+ release in parotid slices of the treated animals, and omission of Ca^{++} from this medium, or the previous addition of lanthanum, did not inhibit K^+ release in these slices as it did in control slices. These findings suggest that both the handling of Na^+ and of Ca^{++} are impaired in the parotid gland after chronic reserpine administration. Because these two ions are intimately involved in the response of α-receptors in the parotid gland (1, 9, 11) this response is impaired after the drug treatment. An impairment in the handling of Ca^{++} probably results in a deficient generation of cyclic GMP and, therefore, in a reduced activation of the Na^+-K^+ pump and of K^+ uptake.

The significant reduction in amylase release observed in parotid slices of reserpine-treated rats most likely results from a deficient level or utilization of energy resources. A similar reduction was observed in control slices when ATP was depleted by removal of

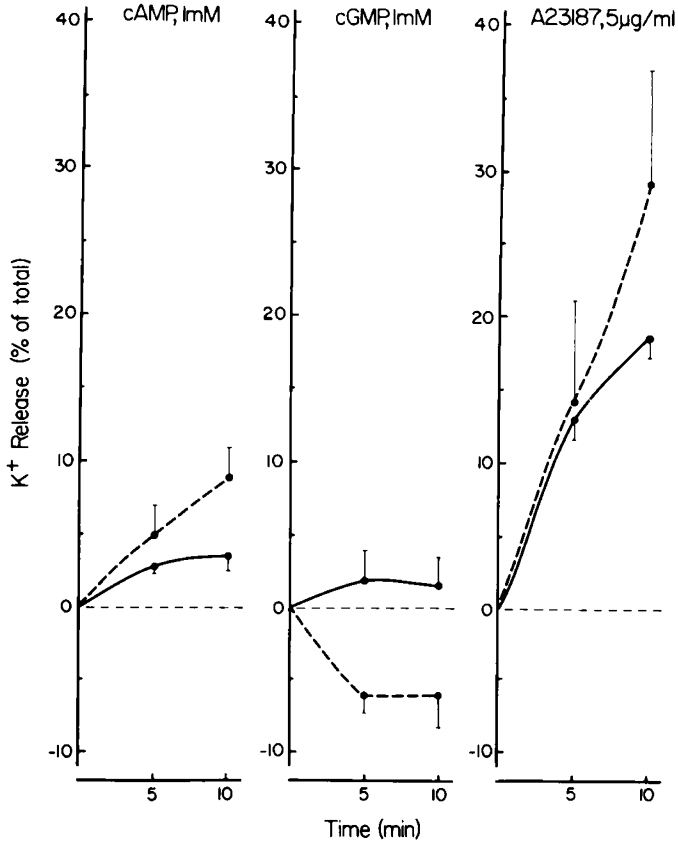

Fig. 5. K^+ release from parotid slices of control and reserpine-treated rats in the presence of the divalent cation ionophore A 23187 (5 µg/ml), the 8-bromo derivative of cyclic GMP (10^{-3} M) and the dibutyryl derivative of cyclic AMP (10^{-3} M). Number of experiments for each case was 4.

Table 3. *Effect of substrate removal on K^+ and amylase release*[1]

Secretagogue	Type of gland	K^+ release[2] (% of total)	Amylase release[3] (% of total)
Epinephrine	Control	21.1 ± 2.8	17.1 ± 1.5
	Reserpine	23.3 ± 6.0	14.0 ± 1.8
Isoproterenol	Control	3.3 ± 0.8	25.6 ± 1.4
	Reserpine	6.9 ± 4.7	10.2 ± 0.7
A 23187	Control	17.6 ± 1.6	12.9 ± 3.5
	Reserpine	22.6 ± 2.3	9.7 ± 0.5
CGMP	Control	8.7 ± 0.3	8.1 ± 0.8
	Reserpine	15.0 ± 0.7	6.7 ± 0.4
cAMP	Control	3.3 ± 0.03	13.5 ± 0.5
	Reserpine	20.2 ± 4.0	9.3 ± 0.8

[1] The purines inosine and adenine were removed from the KRB.
[2] K^+ release after 10 min of incubation.
[3] Amylase release after 30 min of incubation.

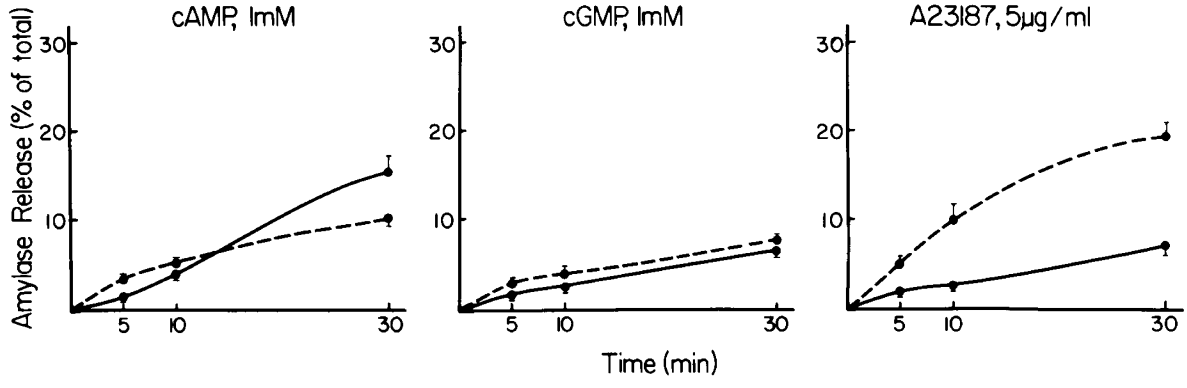

Fig. 6. Amylase release from parotid slices of control and reserpine-treated rats in the presence of the divalent cation ionophore A 23187, 8-bromo cyclic GMP and dibutyryl cyclic AMP, in the same experiments illustrated in Figure 5.

the purines from the incubation medium. It may also involve an impairment of the sequence of events leading to enzyme release, as suggested by the failure of the exogenous derivative of cyclic AMP to cause amylase release in the slices of the treated animals. The lack of dependency of K^+ release on external Ca^{++} in these slices suggests that they have access to an alternate source of Ca^{++} ions, most likely from internal stores. If this is the case, the release of internal Ca^{++} would not be a limiting factor in the mechanism of amylase release. In fact, the availability of internal Ca^{++} may be increased after chronic reserpine administration, because this drug has been shown to make Ca^{++} more available or to increase the efficiency of its utilization in other tissues (7). Our results indicate, therefore, that β-receptor responses are also impaired in the parotid gland after chronic reserpine administration.

The chronic administration of reserpine to rats has been shown to induce abnormalities in several exocrine glands and secretions that resemble those of cystic fibrosis. The present findings in the parotid gland suggest that the secretory defect in this disease may be related to alterations in either the sensitivity of adrenergic receptors or the generation of the intracellular signals leading to the secretion of ions and of macromolecules. In either case, a disturbed stimulus-secretion coupling mechanism would result during the response to physiologic stimulation.

CONCLUSION

After chronic administration of reserpine, slices of the rat parotid gland incubated *in vitro* were found to release significantly more K^+ and significantly less amylase than similar slices from untreated control animals. The extent of K^+ release in the gland slices of the drug-treated animals shows no dependency on external Na^+ or Ca^{++} and is probably enhanced because of a decreased activation of K^+ uptake by the Na^+-K^+ pump. This effect may also involve a defective generation of cyclic GMP, which was found to enhance K^+ uptake and, thus, to inhibit K^+ release in control slices. The inhibition of amylase release observed after treatment with reserpine most likely results from a deficient level or utilization of the energy or nucleotide sources required for enzyme secretion, because a similar inhibition was observed in control slices after incubation in the absence of the purines inosine and adenine. These results indicate that chronic reserpine administration impairs the stimulus-secretion coupling mechanism in the rat parotid gland and results in abnormal responses from both α and β adrenergic receptors.

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