Abstract—Oxygen uptake and amylase output in rat submandibular gland slices were measured by utilizing adrenergic agonists. Adrenaline, noradrenaline and isoproterenol significantly stimulated the oxygen uptake and amylase output. In the presence of propranolol or phenoxybenzamine, adrenaline-stimulated oxygen uptake was obviously blocked. Adrenaline-stimulated amylase output was inhibited by propranolol, but not by phenoxybenzamine. The increase in oxygen uptake by noradrenaline was blocked by phenoxybenzamine, but not by propranolol. Isoproterenol-stimulated oxygen uptake and amylase output were strongly inhibited by propranolol. The oxygen uptake due to isoproterenol was little affected by phenoxybenzamine. These results suggest that the increase in oxygen uptake seen with adrenergic agonists is mediated by both \(\alpha\) and \(\beta\)-receptors, and that the amylase output is evoked through the stimulation of \(\beta\)-receptors.

The term “stimulus-secretion coupling” was originally coined by Douglas and Rubin (1, 2) to describe the sequence of events initiated by acetylcholine (ACh) stimulation of adrenal chromaffin cells and leading to the secretion of catecholamines by exocytosis. They apparently had in mind the close similarity to the phenomenon of “excitation-contraction coupling” in muscle (namely, the key role of calcium in mediating both secretion and contraction and the parallel set of electrical and ionic events at the plasma membrane in response to ACh). We previously reported that the oxygen uptake in dog submandibular gland slices was stimulated by addition of ACh \(10^{-4}-5 \times 10^{-3} \text{ M}\) and K\(^+\) \(14.25-57 \text{ mM}\) (3). Both K\(^+\)- and ACh-stimulated oxygen uptake was inhibited by Ca\(^2+\) omission from the incubation medium, and the reversibility of the effects of K\(^+\) and ACh was recognized by the addition of 5 mM Ca\(^2+\). These results suggested that Ca\(^2+\) plays an important role in the oxygen uptake response to K\(^+\) and ACh of the submandibular gland slices.

Pieces or slices of rat parotid gland, incubated in vitro, respond to adrenaline and carbachol by increasing \(\alpha\)-amylase output and oxygen uptake (4, 10). Adrenaline activates amylase secretion through \(\beta\)-receptors and K\(^+\) release through \(\alpha\)-receptors (5, 6). Phenylephrine increases amylase output through the stimulation of both \(\alpha\) and \(\beta\)-receptors (4, 12). The increasing concentration of cyclic AMP in the acinar cells is involved in the increase of amylase output by adrenaline and phenylephrine. Lindsay et al. (7) reported that \(\alpha\)-amylase secretion from rat parotid tissue in vitro was neither critically dependent on nor closely related to any of the accompanying alterations in tissue metabolism (glucose and
leucine oxidation) and suggested that cyclic AMP appeared to be involved in the control of secretion, but not in the control metabolism.

We studied the mechanism of respiration and amylase output as induced by adrenergic agonists in rat submandibular gland slices.

MATERIALS AND METHODS

Submandibular gland slices were obtained from 200-300 g male rats (Wistar strain) fasted for 18 hr prior to sacrifice. The animals were decapitated. Samples of submandibular gland slices were excised and each gland was divided with a Stadie-Riggs slicer (slice thickness ca. 0.5-0.8 mm). Warburg vessels each containing a slice of approx. 100 mg were immersed in 3 ml of Krebs-Ringer phosphate buffer (pH 7.4). The medium was continuously gassed with 100% O₂ by means of a small polyethylene tube attached to the tissue glass. The oxygen uptake was determined by Warburg's manometric method under pure oxygen for 60 min at 37.5°C. After incubation for 60 min, the amylase output in the medium was determined by the colorimetric method of DyAmyl-L (8, 9). The following agents were used in the experiments: isoproterenol and phenoxybenzamine (Tokyo Kasei Kogyo Co., Ltd.), adrenaline and noradrenaline (Sankyo Co., Ltd.), propranolol (Sumitomo Kagaku Kogyo Co., Ltd.). All the other chemicals obtained commercially were of reagent grade.

RESULTS

Effects of adrenaline, noradrenaline and isoproterenol on the oxygen uptake in rat submandibular gland slices: The oxygen uptake was stimulated by the addition of adrenergic agonists (Figs. 1-3). The oxygen uptake was remarkably induced by adrenaline 5.5 and 55 µM, but was not observed when adrenaline was given in either low and high concentrations. On the other hand, the noradrenaline-induced oxygen uptake was considerably stimulated at 55 µM. Isoproterenol was less effective on the oxygen uptake at low concentrations (1 and 10 µM), but was remarkably stimulated at high concentrations (100 and

![Fig. 1. Effects of various concentrations of adrenaline on the oxygen uptake in rat submandibular gland slices. Submandibular gland slices (approx. 100 mg) were incubated in 3 ml Krebs-Ringer phosphate buffer under pure oxygen for one hour at 37.5°C. Each point represents the mean ± S.E. for seven experiments.](image-url)
Effects of propranolol and phenoxybenzamine on the oxygen uptake induced by adrenaline, noradrenaline and isoproterenol: Effects of propranolol and phenoxybenzamine on adrenergic agonist-induced oxygen uptake are shown in Table 1. Adrenaline-induced oxygen uptake was inhibited by propranolol, a $\beta$-adrenergic antagonist, at 3 and 33 $\mu$M. Similarly, adrenaline-induced oxygen uptake was remarkably inhibited by phenoxybenzamine, an $\alpha$-adrenergic antagonist. Noradrenaline-stimulated oxygen uptake was inhibited by the addition of phenoxybenzamine, but was not inhibited by propranolol. On the other hand, isoproterenol-induced oxygen uptake was markedly inhibited by the addition of propranolol, but was little affected by phenoxybenzamine.

Effects of adrenaline, noradrenaline and isoproterenol on amylase output in rat submandibular gland slices: Amylase output from submandibular gland slices was enhanced by the addition of adrenergic agonists (Table 2). Adrenaline at a low concentration (0.55 $\mu$M) did not stimulate the amylase output from submandibular gland slices, but adrenaline
at elevated concentrations (5.5 and 55 μM) in the medium increased the extent of amylase output. Similarly, both noradrenaline and isoproterenol increased the amylase output. In particular, the amylase output induced by the addition of isoproterenol was markedly

**TABLE 1. Effects of propranolol and phenoxybenzamine on the oxygen uptake induced by adrenaline, noradrenaline and isoproterenol**

| Condition                      | Oxygen uptake (μlO₂/100 mg wet wt./hr) |
|--------------------------------|----------------------------------------|
| Control                        | 121 ± 3                                 |
| +33 μM Propranolol             | 114 ± 7 NS                              |
| +500 μM Phenoxybenzamine       | 126 ± 4 NS                              |
| 5.5 μM Adrenaline              | 190 ± 5                                 |
| -3 μM Propranolol              | 167 ± 1*                                |
| +33 μM Propranolol             | 136 ± 6*                                |
| +500 μM Phenoxybenzamine       | 144 ± 3*                                |
| 55 μM Noradrenaline            | 170 ± 6                                 |
| +3.3 μM Propranolol            | 166 ± 5 NS                              |
| +33 μM Propranolol             | 164 ± 5 NS                              |
| +50 μM Phenoxybenzamine        | 151 ± 3 *                               |
| +500 μM Phenoxybenzamine       | 147 ± 4 *                               |
| 100 μM Isoproterenol           | 173 ± 5                                 |
| +3.3 μM Propranolol            | 145 ± 3 *                               |
| +33 μM Propranolol             | 130 ± 5 *                               |
| +500 μM Phenoxybenzamine       | 178 ± 8 NS                              |

NS; Not significant. *p<0.001 compared with the corresponding value with adrenaline, noradrenaline or isoproterenol. Experimental conditions were as described in Fig. 1. Each value represents the mean ± S.E. for seven experiments.

**TABLE 2. Effects of adrenaline, noradrenaline and isoproterenol on the amylase output in rat submandibular gland slices**

| Conditions                      | Amylase output (Unit Somogyi/100 mg wet wt./hr) |
|--------------------------------|-----------------------------------------------|
| Control                        | 4.16 ± 0.17                                   |
| 0.55 μM Adrenaline             | 4.80 ± 0.17 NS                               |
| 5.5 μM Adrenaline              | 6.14 ± 0.17 *                                |
| 55 μM Adrenaline               | 6.41 ± 0.31 *                                |
| 0.55 μM Noradrenaline          | 5.37 ± 0.20 NS                               |
| 5.5 μM Noradrenaline           | 5.39 ± 0.32 NS                               |
| 55 μM Noradrenaline            | 6.03 ± 0.28 *                                |
| 4 μM Isoproterenol             | 5.72 ± 0.17 *                                |
| 40 μM Isoproterenol            | 8.89 ± 0.42 *                                |

NS; Not significant. *p<0.001 compared with the corresponding value with control. Experimental conditions were as described in Fig. 1. Each value represents the mean ± S.E for seven experiments.
increased and was about 2.1 times the control value at 40 μM of isoproterenol concentration.

Effects of propranolol and phenoxybenzamine on the amylase output induced by adrenaline and isoproterenol: Neither propranolol nor phenoxybenzamine had an effect on the basal amylase output from submandibular gland slices (Table 3). Adrenaline and isoproterenol induced amylase output were significantly inhibited by the addition of propranolol. Propranolol at a high concentration (300 μM) remarkably inhibited the increased amylase output induced by isoproterenol. On the other hand, phenoxybenzamine, α-adrenergic blocking agent, considerably increased the amylase output induced by adrenaline.

**DISCUSSION**

When both K⁺ and ACh are added to dog submandibular gland slices, oxygen uptake is initiated (3). Putney (10) reported that carbachol, substance P and adrenaline transiently stimulated the oxygen uptake by 40-50% in rat parotid gland slices, while this effect was not seen with isoproterenol. The increase in the oxygen uptake due to carbachol was blocked by atropine. These results show that the oxygen uptake in the parotid gland slices is stimulated following activation of muscarinic, adrenergic or peptide receptors. In the present experiment, the addition of adrenaline and noradrenaline to the segments of rat submandibular gland slices increased the oxygen uptake and amylase output, and the response to adrenaline was higher than that to noradrenaline. The oxygen uptake and amylase output were also stimulated by isoproterenol.

Hagen (11) found that the increase in oxygen uptake due to adrenaline was blocked by dibenamine, an α-adrenergic blocking agent, in rat parotid gland slices. In the present experiment, adrenaline-induced oxygen uptake was blocked by phenoxybenzamine. These results are consistent with those obtained by Hagen (11). Further, adrenaline-induced oxygen uptake was also blocked by propranolol, a β-adrenergic antagonist. On the other
hand, noradrenaline-induced oxygen uptake was inhibited by the addition of phenoxybenzamine, but was not by propranolol. These observations indicate that noradrenaline has an $\alpha$-effect in submandibular gland slices. Isoproterenol-induced oxygen uptake was remarkably inhibited by propranolol, but was inhibited little by phenoxybenzamine. These results suggest that increase in the oxygen uptake seen with adrenergic agonists is mediated by both $\alpha$- and $\beta$-receptors in rat submandibular gland slices.

The amylase output in rat and mouse parotid glands is stimulated by ACh and carbachol, but the stimulation of cholinergic agonists is considerably low compared to that of adrenergic agonists (12, 13). Cholinergic agonist-induced amylase output is inhibited by the addition of atropine (12). Adrenaline evokes in rat parotid slices two apparently independent processes: amylase output and $K^+$ release (14). Batzri and Selinger (5) reported that adrenaline activates the amylase output through $\beta$-adrenergic receptors and $K^+$ release through $\alpha$-adrenergic receptors. The increase in $K^+$ release due to adrenaline is not mediated by changes in the concentration of cyclic AMP in the cells. On the other hand, the secretory process of amylase is mediated by cyclic AMP (4, 5, 12, 15). In the present experiment, the adrenaline stimulation of the amylase output was abolished by the addition of propranolol. However, phenoxybenzamine, $\alpha$-adrenergic blocking agent, did not abolish the amylase output. On the other hand, isoproterenol-stimulated amylase output was also inhibited by the addition of propranolol. These results suggest that increase in the amylase output due to adrenaline and isoproterenol is evoked by stimulating $\beta$-receptors in rat submandibular gland slices.

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