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# Inhibitory regulation of amylase release in rat parotid acinar cells by benzodiazepine receptors

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**Abstract**

This study examined the influence of benzodiazepine receptors on amylase release from rat parotid acinar cells. Diazepam ($10^{-8}$–$10^{-6}$ M), which is a potent agonist of both central- and peripheral-type benzodiazepine receptors, dose dependently decreased amylase release induced by isoprenaline and carbachol, which are β-adrenoceptor and muscarinic receptor agonists, respectively. The maximum inhibitory response was obtained with $10^{-6}$ M diazepam: amylase release was decreased to 57% (isoprenaline) and 39% (carbachol) of maximal levels, while these responses were completely inhibited by propranolol and atropine, respectively. Clonazepam and 7-chloro-1,3-dihydro-1-methyl-5-( *p*-chlorophenyl)-2H-1,4-benzodiazepine-2-one (Ro 5-4864), which are selective agonists of central- and peripheral-type benzodiazepine receptors, respectively, also produced a significant and dose-dependent decrease in isoprenaline-induced amylase release. The inhibitory potency was diazepam > clonazepam > Ro 5-4864. Flumazenil and 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide (PK 11195), which are selective antagonists of central- and peripheral-type benzodiazepine receptors, respectively, dose dependently blocked the inhibition of isoprenaline-induced amylase release by diazepam. At a concentration of $10^{-5}$ M, flumazenil and PK 11195 restored amylase release to ~75% of that in the presence of isoprenaline alone. The combination of both antagonists completely prevented the inhibition by diazepam. Similarly, the inhibitory responses of clonazepam and Ro 5-4864 were completely blocked by flumazenil and PK 11195, respectively. These results suggest that, in rat parotid acinar cells, benzodiazepines inhibit β-adrenoceptor and muscarinic receptor-stimulated amylase release and that both central- and peripheral-type benzodiazepine receptors contribute to this inhibitory regulation. 

*Keywords:* Benzodiazepine; Amylase release; Benzodiazepine receptor; Parotid acinar cell

## 1. Introduction

Benzodiazepines such as diazepam, clonazepam, and nitrazepam have hypnotic, anxiolytic, and anticonvulsant actions mediated via the central-type benzodiazepine receptors in the central nervous system (Möhler and Okada, 1977; Skolnick and Paul, 1982; Greenblatt et al., 1983; Bormann, 1988). On the other hand, these drugs have been known to induce xerostomia as a serious oral side effect (Sreebny and Schwartz, 1986).

Benzodiazepine receptors have been classified into two types: a central-type linked to the $GABA_A$ receptor–chloride channel complex and a peripheral-type not linked to the $GABA_A$ receptor (Braestrup and Squires, 1977; Schoemaker et al., 1983; Rampe and Triggle, 1986). The distribution of peripheral-type benzodiazepine receptors has been reported in several peripheral tissues, including the kidney, heart, lung, pancreas, and adrenal glands (Davies and Huston, 1981; Anholt et al., 1986; Giusti et al., 1994). Autoradiographic localization of peripheral-type benzodiazepine receptors was successful in rat salivary glands (De Souza et al., 1985). Binding studies suggest that both central- and peripheral-type benzodiazepine receptors exist in rat salivary glands as well as in the brain (Kawaguchi and Yamagishi, 1996; Yamagishi and Kawaguchi, 1998). Moreover, it has been demonstrated that GABA and its biosynthetic and metabolic enzymes exist in rat salivary glands (Sawaki et al., 1995). We previously reported that diazepam produced a decrease in pilocarpine-stimulated salivary secretion in the rat and that, in rat parotid acinar cells, diazepam increased $^{36}Cl^-$ influx and decreased $^{36}Cl^-$ efflux. These actions were enhanced by GABA and muscimol, a $GABA_A$ receptor agonist, and inhibited by $GABA_A$ and benzodiazepine receptor antagonists (Kawaguchi et al., 1995; Kawaguchi and Yamagishi, 1996). These findings

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strongly suggest that benzodiazepines not only suppress the central nervous system but also act directly on the salivary glands and that the benzodiazepine and GABA receptors may contribute to benzodiazepine-induced xerostomia.

Amylase in the salivary glands is mainly released through the intracellular cyclic AMP pathway by β-adrenoceptor stimulation. Fluid secretion is induced through the phosphoinositide pathway by muscarinic and α-adrenoceptor stimulation (Butcher and Putney, 1980; Baum, 1987). Benzodiazepines inhibit dopamine release and glucose-induced insulin secretion in peripheral tissues (Ohara-Imaizumi et al., 1991; Petit et al., 1992). However, the functional role of benzodiazepine receptors in amylase release from rat parotid acinar cells has not yet been clearly established. Consequently, the aim of the present study was to examine whether benzodiazepines modify the release of amylase induced by β-adrenoceptor and muscarinic receptor agonists. To this purpose we tested specific agonists and antagonists for the central- and peripheral-type benzodiazepine receptors.

## 2. Materials and methods

### 2.1. Animals

Male Wistar strain rats, 200–250 g, were purchased from Japan SLC (Hamamatsu, Japan). The animals were housed in an air-conditioned room (temperature $23 \pm 2$°C, humidity $55 \pm 5$%, and lighting 6:00 AM–6:00 PM) and maintained on commercial laboratory chow and water ad libitum for one week before the experiments.

### 2.2. Chemicals and medium

Isoprenaline, propranolol, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES), bovine serum albumin, carbamylcholine chloride (carbachol), bovine testicular hyaluronidase (type 1-S), and calf thymus DNA (type 1) were purchased from Sigma (St. Louis, MO, USA). Collagenase (CLSPA) was purchased from Worthington Biochemical (Freehold, NJ, USA). Diazepam, atropine, diphenylamine and 3,5-dinitrosalicylic acid were purchased from Wako (Osaka, Japan). 7-Chloro-1,3-dihydro-1-methyl-5-(*p*-chlorophenyl)-2H-1,4-benzodiazepine-2-one (Ro 5-4864) was provided by Hoffmann-La Roche (Nutley, NJ, USA). 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide (PK 11195) was purchased from Research Biochemicals International (Natick, MA, USA). Dulbecco's Modified Eagle Medium (DMEM) and Hanks' balanced salt solution (HBSS) were obtained from Gibco BRL (Grand Island, NY, USA). Dimethyl sulfoxide (DMSO) was obtained from Nacalai Tesque (Kyoto, Japan). Flumazenil and clonazepam were kindly provided by Yamanouchi Pharmaceuticals (Tokyo, Japan) and Sumitomo Pharmaceuticals (Osaka, Japan), respectively. All other reagents used were of the highest grade commercially available. Benzodiazepines and receptor-related agents were dissolved in DMSO, which had no effect at the concentration used in this study. β-Adrenoceptor and muscarinic receptor-related agents were dissolved in distilled water. The 'digestion medium' used for the acinar cell preparation was DMEM containing 1% bovine serum albumin, collagenase (100 U/ml) and hyaluronidase (0.15 mg/ml). HBSS-H was HBSS containing 1.27 mM $CaCl_2$, 0.81 mM $MgSO_4$ and 30 mM HEPES (pH 7.4).

### 2.3. Preparation of parotid acinar cells

Parotid acinar cells were prepared as described by Melvin et al. (1987) with some modifications. Briefly, parotid glands were immediately removed after cardiac puncture with ether anesthesia, dissected free of fat, connective tissues, and lymph nodes, and finely minced in the digestion medium (5 ml/animal). The minced glands were incubated for 1 h at 37°C with continuous agitation at 100 cycles/min under an atmosphere of 95% $O_2$/5% $CO_2$. At 20-min intervals, the mince was dispersed by gentle pipetting and gassed. After enzyme digestion, the resulting cell aggregates were washed three times with HBSS-H containing bovine serum albumin (0.1 mg/ml) and further incubated for 20 min at 37°C in the same medium (7 ml/animal). The acinar cell aggregates obtained were filtered through a Nytex nylon screen (105 $\mu m^2$) and suspended in fresh HBSS-H (7 ml/animal). The cells obtained were $> 90$% living cells. The cell preparations were usually made between 9:00 AM and 11:30 AM to avoid circadian variations in amylase activity.

### 2.4. Study of amylase release

One milliliter of acinar cell suspension was preincubated for 5 min at 37°C with benzodiazepines or the receptor-related agents and further incubated for 30 min with isoprenaline or carbachol and continuous agitation under 95% $O_2$/5% $CO_2$. After incubation, the assay medium was filtered through a glass microfiber filter (Whatman GF/C). To measure the total amylase activity in the cell suspension, a portion of the suspension was homogenized with a polytron homogenizer and filtered similarly. The filtrates obtained and the acinar cells on the filter were used for amylase activity measurements and DNA analysis, respectively. All assays were performed in duplicate.

### 2.5. Measurement of amylase activity and DNA content

Amylase activity was assayed according to the method of Bernfeld (1955) with some modifications. One hundred microliters of diluted enzyme solution was incubated for 5

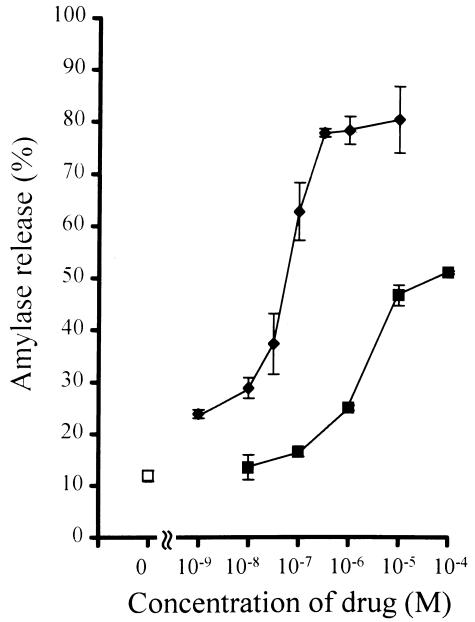


Fig. 1. Dose–response curves for amylase release induced by isoprenaline and carbachol in rat parotid cells. Rat parotid acinar cells were incubated for 30 min with the indicated concentrations of isoprenaline (♦) or carbachol (■). Then the released amylase was measured and expressed as a percentage of total amylase activity of the cells. Open squares show control values. Control amylase activity was $0.35 \pm 0.01$ mg maltose/μg DNA. The total amylase activity was $3.27 \pm 0.03$ mg maltose/μg DNA. Data are means ± S.E.M. of three to seven experiments.

min at 25°C with 100 μl of 1% starch solution in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM NaCl. The enzyme reaction was stopped by the addition of 200 μl of 1% 3,5-dinitrosalicylic acid in 0.4 M NaOH containing 30% Rochelle salt. Then this mixture was heated for 5 min in boiling water. After addition of 5 ml of distilled water, the absorption of the solution was determined photometrically at 530 nm. A calibration curve established with maltose was used to convert the colorimeter readings. Amylase activity was expressed as mg maltose liberated per microgram of DNA. DNA analysis was carried out by the diphenylamine method (Richards, 1974), with calf thymus DNA as the standard. The released amylase is expressed as a percentage of the total amylase activity in the parotid acinar cells.

### 2.6. Data analysis

The data are presented as the means ± S.E.M. The analyses were performed by Student's *t*-test or Dunnett's multiple comparison test. The difference in means was considered statistically significant when $P$ values were less than 0.05.

## 3. Results

### 3.1. Effect of β-adrenoceptor and muscarinic receptor agonists on amylase release

In rat parotid acinar cells, the concentration–response curves for amylase release induced by isoprenaline and carbachol are shown in Fig. 1. In concentrations ranging from $10^{-9}$ M to $10^{-5}$ M, isoprenaline significantly increased amylase release in a concentration-dependent manner. Similarly, carbachol, in concentrations ranging from $10^{-8}$ M to $10^{-4}$ M, dose dependently increased amylase release. The maximum responses were obtained with $10^{-5}$

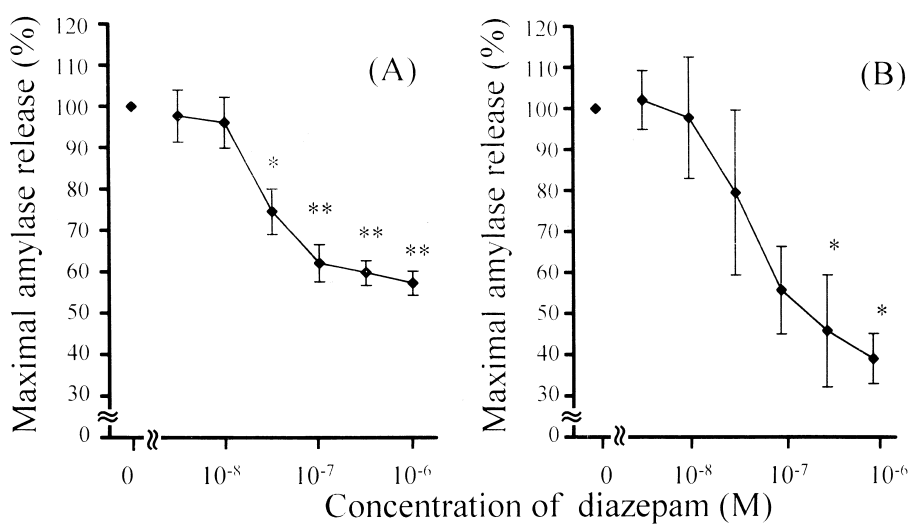


Fig. 2. Effects of diazepam on amylase release induced by isoprenaline (A) and carbachol (B) in rat parotid cells. Rat parotid acinar cells were preincubated for 5 min with the indicated concentrations of diazepam and further incubated for 30 min with isoprenaline ($10^{-5}$ M) or carbachol ($10^{-5}$ M). Then the released amylase was measured and expressed as a percentage of amylase release induced by isoprenaline or carbachol alone. The activity of amylase released by isoprenaline and carbachol alone was $3.86 \pm 0.51$ and $2.46 \pm 0.42$ mg maltose/μg DNA, respectively. The total amylase activity was $4.87 \pm 0.39$ mg maltose/μg DNA. Data are means ± S.E.M. of three to five experiments. *, **Significantly different from isoprenaline or carbachol alone at $P < 0.05$ and $P < 0.01$, respectively.

M isoprenaline and $10^{-4}$ M carbachol. The maximal release was 80.9% and 51.5%, respectively. The response elicited by the β-adrenoceptor agonist was about 1.6 times that elicited by the muscarinic receptor agonist at a concentration of $10^{-5}$ M. Thus, in the subsequent amylase release experiments, isoprenaline and carbachol were used at concentrations of $10^{-5}$ M.

### *3.2. Effect of benzodiazepine receptor agonists on amylase release*

Diazepam has a high affinity for both central- and peripheral-type benzodiazepine receptors. We first examined the effect of diazepam on amylase release (Fig. 2). Diazepam significantly decreased isoprenaline- and carbachol-induced amylase release in a concentration-dependent manner. The maximum decrease was obtained with $10^{-6}$ M diazepam, and isoprenaline- and carbachol-induced amylase release decreased to 57.4% or 39.2% of their maximal levels, respectively. However, the decrease in the specific activity of amylase was approximately the same (1.64 mg maltose/μg DNA for isoprenaline and 1.50 mg maltose/μg DNA for carbachol). No significant change in amylase release was observed with diazepam treatment alone without the agonists (data not shown). We also examined the effects of the other benzodiazepine receptor

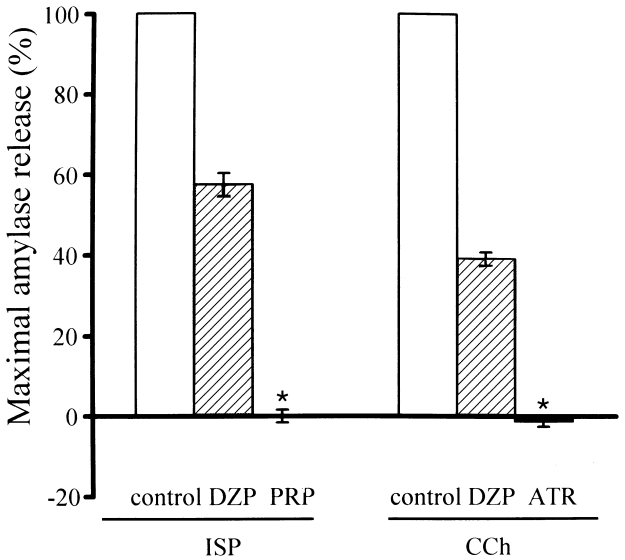


Fig. 4. Effects of β-adrenoceptor and muscarinic receptor antagonists on isoprenaline- and carbachol-induced amylase release in rat parotid cells. Rat parotid acinar cells were preincubated for 5 min with propranolol (PRP; $10^{-4}$ M) or atropine (ATR; $10^{-5}$ M) and further incubated for 30 min with isoprenaline (ISP; $10^{-5}$ M) or carbachol (CCh; $10^{-5}$ M). Then the released amylase was measured and expressed as a percentage of amylase release induced by ISP or CCh alone. The data for diazepam (DZP; $10^{-6}$ M) are taken from Fig. 2. Data are means ± S.E.M. of three to six experiments. * Significantly different from inhibition by DZP at $P < 0.01$.

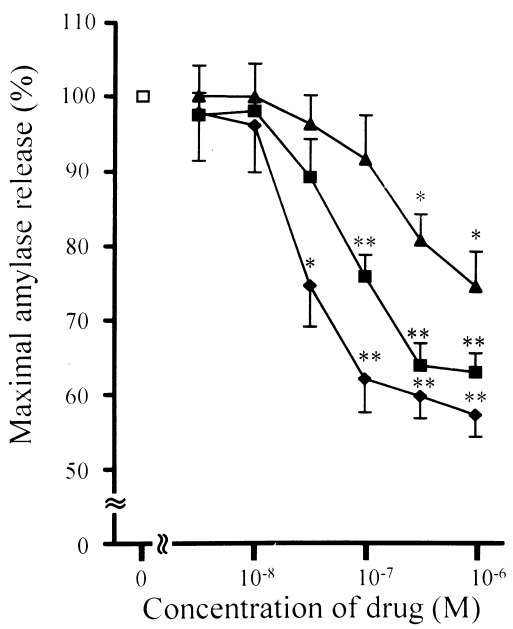


Fig. 3. Effects of benzodiazepine receptor agonists on amylase release induced by isoprenaline in rat parotid cells. Rat parotid acinar cells were preincubated for 5 min with the indicated concentrations of diazepam (♦), clonazepam (■), and Ro 5-4864 (▲), respectively, and further incubated for 30 min with isoprenaline ($10^{-5}$ M). Then the released amylase was measured and expressed as a percentage of amylase release induced by isoprenaline alone (□). Data are means ± S.E.M. of four to six experiments. *, ** Significantly different from isoprenaline alone at $P < 0.05$ and $P < 0.01$, respectively.

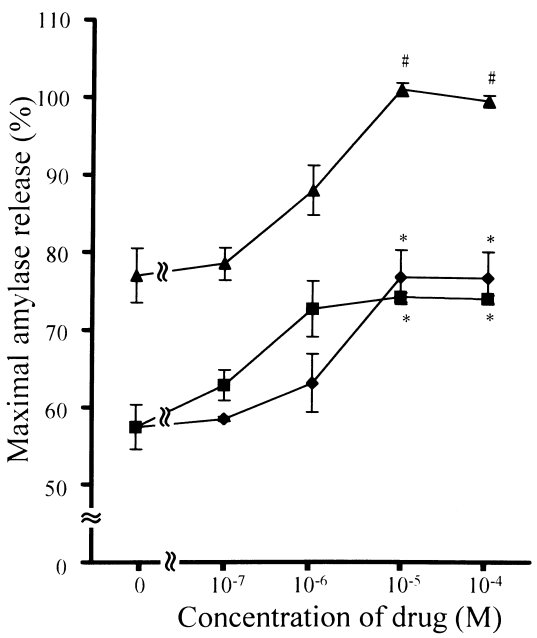


Fig. 5. Effects of benzodiazepine receptor antagonists on inhibition of isoprenaline-induced amylase release by diazepam in rat parotid cells. Rat parotid acinar cells were preincubated for 5 min with diazepam ($10^{-6}$ M) and the indicated concentrations of flumazenil (♦), PK 11195 (■), or flumazenil ($10^{-5}$ M) plus the indicated concentrations of PK 11195 (▲), and further incubated for 30 min with isoprenaline ($10^{-5}$ M). Then the released amylase was measured and expressed as a percentage of amylase release induced by isoprenaline alone. Data are means ± S.E.M. of three to seven experiments. *, # Significantly different from diazepam alone or flumazenil ($10^{-5}$ M) plus diazepam at $P < 0.01$, respectively.

Table 1
Effects of benzodiazepine receptor antagonists on inhibition of isoprenaline-induced amylase release by Ro 5-4864 and clonazepam in rat parotid cells

| Drugs | Maximal amylase release (%) |
|---|---|
| Ro 5-4864 ($10^{-6}$ M) | 74.8 ± 4.7 |
| + PK 11195 ($10^{-5}$ M) | 98.5 ± 3.3[a] |
| + Flumazenil ($10^{-5}$ M) | 76.1 ± 10.0 |
| Clonazepam ($10^{-6}$ M) | 63.3 ± 2.5 |
| + PK 11195 ($10^{-5}$ M) | 57.1 ± 3.8 |
| + Flumazenil ($10^{-5}$ M) | 95.6 ± 1.6[b] |
| Diazepam ($10^{-6}$ M) | 57.4 ± 2.9 |
| + PK 11195 ($10^{-5}$ M) | 74.3 ± 0.6[b] |
| + Flumazenil ($10^{-5}$ M) | 77.0 ± 3.5[b] |
| + PK 11195 ($10^{-5}$ M) + flumazenil ($10^{-5}$ M) | 101.4 ± 0.9[b] |

Rat parotid acinar cells were preincubated for 5 min with the indicated concentrations of Ro 5-4864, clonazepam, PK 11195, or flumazenil and further incubated for 30 min with isoprenaline ($10^{-5}$ M). Then the released amylase was measured and expressed as a percentage of amylase release induced by isoprenaline alone. The data for diazepam are taken from Fig. 5. Data are means ± S.E.M. of three to seven experiments.
[a] $P < 0.05$; [b] $P < 0.01$, compared to benzodiazepine receptor agonist alone.

agonists and compared them with that of diazepam (Fig. 3). In a manner similar to diazepam, clonazepam, a selective central-type agonist, and Ro 5-4864, a selective peripheral-type agonist, also produced a significant and dose dependent decrease in the amylase release elicited by isoprenaline. At concentrations of $10^{-6}$ M, clonazepam and Ro 5-4864 decreased amylase release to 63.2% and 74.8% of the levels measured after incubation with isoprenaline alone, respectively. The inhibitory potency on amylase release was diazepam > clonazepam > Ro 5-4864.

### *3.3. Effect of β-adrenoceptor and muscarinic receptor antagonists on amylase release*

Unlike the benzodiazepines, propranolol, a β-adrenoceptor antagonist, and atropine, a muscarinic receptor antagonist, completely inhibited isoprenaline- and carbachol-induced amylase release (Fig. 4).

### *3.4. Effect of benzodiazepine receptor antagonists on amylase release*

To determine the specificity of the benzodiazepine receptor in the inhibitory response, we further examined the effect of benzodiazepine receptor antagonists (Fig. 5). Flumazenil, a selective central-type antagonist, and PK 11195, a selective peripheral-type antagonist, significantly blocked the inhibition of isoprenaline-induced amylase release by diazepam in a concentration-dependent manner. At concentrations of $10^{-5}$ M, both antagonists restored amylase release from 57.4% to 77.0% and 74.4% of levels measured after incubation with isoprenaline alone, respectively. Complete blockade was not observed with either antagonist alone. However, when both antagonists were present simultaneously, the inhibitory response to diazepam was completely blocked (Fig. 5 and Table 1). Flumazenil also blocked the inhibitory response to clonazepam, but not to Ro 5-4864, and significantly restored amylase release to 95.6% of levels measured after incubation with isoprenaline alone (Table 1). Similarly, PK 11195 completely blocked the inhibitory response to Ro 5-4864, but not to clonazepam (Table 1).

## 4. Discussion

The present results show clearly that benzodiazepines inhibit amylase release from rat parotid acinar cells induced by β-adrenoceptor and muscarinic receptor stimulation, and that the benzodiazepine receptors in parotid membranes are likely associated with these inhibitory responses.

Amylase release from the parotid cells through the intracellular cyclic AMP pathway is mainly induced by β-adrenoceptor stimulation. A smaller amount of amylase is released by muscarinic $m_3$-receptor and $\alpha_1$-adrenoceptor stimulation, which mainly causes fluid secretion through the phosphoinositide pathway (Butcher and Putney, 1980; Baum, 1987). In the present experiments, we reproduced both of these amylase release responses. At low concentrations ($< 10^{-8}$ M), isoprenaline, but not carbachol, significantly increased amylase release, and the stimulatory response of isoprenaline was more potent than that of carbachol (Fig. 1). These results are consistent with those reported by other investigators (Pohto, 1968; Kanagasuntheram and Randle, 1976).

Central- and peripheral-type benzodiazepine receptors are found in several peripheral tissues, including the heart, pancreas, and adrenal glands (Davies and Huston, 1981; Anholt et al., 1986; Giusti et al., 1994). Central-type benzodiazepine receptors are located on the plasma membrane. Conversely, peripheral-type benzodiazepine receptors are localized typically in the mitochondrial outer membrane but can also be associated with non-mitochondrial (including plasma) membranes (Anholt et al., 1986; Olson et al., 1988; O'Beirne et al., 1990; Woods and Williams, 1996). In the salivary glands, the existence of both central- and peripheral-type benzodiazepine receptors has been clearly demonstrated in crude parotid membranes (Kawaguchi and Yamagishi, 1996; Yamagishi and Kawaguchi, 1998). However, the exact subcellular location(s) of the peripheral-type benzodiazepine receptors in this exocrine gland has not yet been established. Thus, conceivably, both types of benzodiazepine receptors could be present in the same cell membrane.

The central-type benzodiazepines produce hypnotic, anxiolytic, and anticonvulsant actions after interacting with

the central-type benzodiazepine receptor. Actions of the peripheral-type benzodiazepines have been described in a variety of biological systems, some being direct effects, while others are indirect and modulate the actions of other substances (Verma and Snyder, 1989). A physiologic role of mitochondrial benzodiazepine receptors in steroidogenesis and on the release of $Ca^{2+}$ from heart and kidney mitochondria is now recognized (Moreno-Sanchez et al., 1991; Krueger and Papadopoulos, 1993). An exact role for non-mitochondrial benzodiazepine receptor-mediated effects in several other peripheral tissues has not yet been clearly established (Woods and Williams, 1996).

As shown in Figs. 2 and 3, the agonists of central- and peripheral-type benzodiazepine receptors dose dependently decreased isoprenaline- and carbachol-induced amylase release from rat parotid cells. When we compared the inhibitory response to diazepam to the maximal amylase release, the decrease in amylase release caused by carbachol was greater than that caused by isoprenaline (61% and 43%) (Fig. 2). However, when we calculated the specific activity of amylase released by isoprenaline and carbachol, the specific activity was decreased to approximately the same value (1.64 and 1.50 mg maltose/μg DNA). This result indicates that diazepam has the same inhibitory effect on amylase release elicited by isoprenaline or carbachol. At their maximally effective concentrations, none of the three benzodiazepine agonists used were completely able to inhibit amylase release stimulated by either isoprenaline or carbachol, whereas amylase release was completely inhibited by the β-adrenoceptor and muscarinic receptor antagonists, propranolol and atropine (Fig. 4).

These results strongly suggest that the inhibitory effect of benzodiazepines on amylase release may be mediated through central- and peripheral-type benzodiazepine receptors in parotid membranes, but not through β-adrenoceptor and muscarinic receptors. Half-maximal concentrations for causing inhibition of amylase release for diazepam, clonazepam, and Ro 5-4864 were approximately $2.5 \times 10^{-8}$ M, $5.8 \times 10^{-8}$ M and $1.3 \times 10^{-7}$ M, respectively (Fig. 3). Both diazepam and clonazepam inhibited amylase release at a similar concentration to that of their binding affinity for parotid central-type benzodiazepine receptors, namely $18 \times 10^{-8}$ M and $1.86 \times 10^{-8}$ M, respectively (Yamagishi and Kawaguchi, 1998). The half-maximal inhibitory concentration of Ro 5-4864 for amylase secretion is relatively high, $\sim 300 \times$ that of the $K_i$ value (Yamagishi and Kawaguchi, 1998). However, it is not unusual for the functional inhibitory concentration of a drug to be different from its concentration for binding to a receptor (Möhler and Okada, 1977). The inhibitory effect of diazepam is probably the most pronounced because the drug binds to both central- and peripheral-type benzodiazepine receptors. Clonazepam is relatively specific for central-type benzodiazepine receptors, while Ro 5-4864 is relatively specific for peripheral-type benzodiazepine receptors. The results shown in Fig. 3 suggest that both parotid benzodiazepine receptor types contribute to these inhibitory responses.

Ro 5-4864 and PK 11195 were originally described as an agonist and an antagonist, respectively, of the peripheral-type benzodiazepine receptor (Gavish et al., 1992). However, other studies have shown that Ro 5-4864 and PK 11195 produce similar effects. (Grupp et al., 1987; Barnea et al., 1989). There is no explanation at this time for the action of PK 11195. Our suggestions for a modulatory role of benzodiazepine receptors on amylase release were also supported by the results of additional experiments using benzodiazepine receptor antagonists (Fig. 5 and Table 1) in that: (a) flumazenil, a central-type antagonist, and PK 11195, a peripheral-type antagonist, restored isoprenaline-induced amylase release decreased by diazepam to 74–77% of the maximal amylase release; (b) the decreased amylase release was completely restored by a combination of flumazenil and PK 11195; (c) the recoverable response with flumazenil, at high concentrations ($> 10^{-5}$ M), was greater than that with PK 11195; (d) flumazenil blocked the inhibitory response to clonazepam, but not to Ro 5-4864; and (e) PK 11195 blocked the inhibitory response to Ro 5-4864, but not that to clonazepam.

We have previously found that, in vivo and in vitro studies, diazepam produces a decrease in saliva secretion in the rat and modifies $^{36}Cl^-$ transport, which triggers the process of fluid secretion. These actions are inhibited by benzodiazepine receptor antagonists (Kawaguchi et al., 1995; Kawaguchi and Yamagishi, 1996). These findings strongly suggest that an inhibitory mechanism regulating saliva secretion through the central- and peripheral-type benzodiazepine receptors exists in the salivary glands. Our present results, demonstrating that benzodiazepines act directly on rat parotid acinar cells and inhibit amylase release through benzodiazepine receptors, strongly support this hypothesis. The exact mechanism by which bezodiazepines inhibit amylase secretion is not clear. Possible mechanisms involved are (i) that $Cl^-$ influx via a $GABA_A$ receptor coupled to the central-type benzodiazepine receptor could influence intracellular signaling factors involved in amylase release, such as adenylate cyclase, GTP-dependent regulatory proteins (G proteins), cytosolic $Ca^{2+}$ concetration, and protein kinase A (e.g., Deterre et al., 1983; Higashijima et al., 1987); (ii) that topographical changes in the benzodiazepine receptors may occur in association with an increase in the intracellular cAMP concentration (Boujrad et al., 1996); or (iii) that benzodiazepine-induced interactions with clathrin-coated vesicles (Tehrani et al., 1997) or annexins (Hofmann et al., 1998) may perturb the exocytotic routing pathway. Further experiments clearly are required to elucidate these mechanisms.

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