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Biochemical Pharmacology, Vol. 40, No. 2, pp. 390–393, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Effects of indomethacin and prostaglandin E_2 on amylase secretion by rat parotid tissue

(*Received* 13 *July* 1989; *accepted* 12 *February* 1990)

There is much evidence that prostaglandins (PGs) modulate sympathetic [1, 2] and parasympathetic [3–7] neuronal activity. The salivary glands are innervated by both autonomic nervous systems, and excitations of both systems enhance their secretory responses. $PGF_{2\alpha}$ [8–11] and PGE_2 [12] were reported to induce salivary secretion by the submandibular gland of dogs, and PGE_1 [13] to induce secretion by the parotid gland of rats. Results have suggested that $PGF_{2\alpha}$ induces salivation by exciting the parasympathetic neurons [8–11], and that PGE_1 acts as a modulator of the secretory response to cholinergic stimulation [14]. There is also a negative report on the role of endogenous PGs during parasympathetic nerve stimulation of rat salivary glands [15]. However, little is known about whether PGs have primary or modulatory roles in the amylase secretory response of the parotid gland. In the present study we examined the effects of PGE_2 on amylase secretion from rat parotid tissue *in vitro* induced by adrenergic agonists.

Materials and Methods

Parotid glands were obtained from male Wistar rats (250–350 g), and small pieces of the tissue were prepared as described previously [16]. Before experiments, Krebs–Ringer Tris (KRT) solution, consisting of 120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, 3.0 mM $CaCl_2$, 16 mM Tris–HCl buffer (pH 7.4) and 5 mM glucose, was aerated with O_2 gas, and pieces of parotid tissue were equilibrated with the solution for 20 min at 37° with shaking. Samples of about 30 mg of tissue pieces were incubated in 10 mL of KRT solution at 37°, and cumulative secretion of amylase into the medium was measured as described by Bernfeld [17]. Activity was assayed at 20° for 5 min with amylase as substrate and expressed as the amount of maltose liberated into the medium in mg per 100 mg tissue.

Indomethacin was dissolved in ethanol and added to the incubation medium at a final concentration of 14 μM with 0.5% ethanol. This concentration of ethanol did not affect

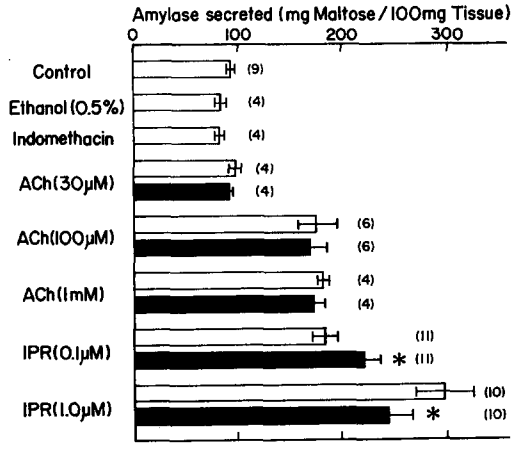

Fig. 1. Effects of indomethacin on amylase secretion from rat parotid tissue. Tissues were incubated at 37° for 10 min with the indicated agonists in the absence (open columns) and presence (closed columns) of 14 μM indomethacin. Columns and bars are means and standard errors; the numbers of experiments are shown in parentheses. Significantly different from the value in the absence of indomethacin by Student's paired *t*-test, $^*P < 0.01$.

amylase secretion. PGE_2 was a gift from the Ono Pharmaceutical Co., Osaka, Japan. Student's paired *t*-test was used to evaluate the significance of differences; a value of $P < 0.05$ was regarded as significant.

Results and Discussion

Indomethacin, an inhibitor of cyclooxygenase, at 14 μM did not have any significant effect on spontaneous amylase secretion from rat parotid tissue or that induced by 30 μM–1 mM ACh (Fig. 1). It also did not affect the secretion induced by α-adrenergic agonists: that is, the secretory responses induced by incubation for 10 min with 100 μM methoxamine in the absence and presence of indomethacin were 143.9 ± 15.0 and 143.7 ± 12.3 mg maltose/100 mg tissue (N = 4), respectively, and those induced by incubation with 100 μM phenylephrine in the absence and presence of indomethacin were 185.8 ± 7.6 and 190.9 ± 4.4 (N = 4), respectively. However, indomethacin increased the secretion induced by 0.1 μM isoproterenol (IPR) and decreased the secretion induced by 1.0 μM IPR (Fig. 1). Thus, it seems likely that indomethacin specifically affected the secretory response mediated through β-adrenoceptors. Next the relation of the effect of indomethacin to the concentration of IPR was studied. The stimulatory effects of IPR in the presence of indomethacin were larger at 0.03 to 0.1 μM and smaller at 1.0 μM than those in its absence, and the two concentration–response curves crossed at IPR concentrations between 0.1 and 1.0 μM (Fig. 2). A stimulatory effect on 0.1 μM IPR-induced secretion and an inhibitory effect on 1.0 μM IPR-induced secretion were obtained within 5 min (not shown) and were clearer after incubation for 20 min (Fig. 2), because IPR-induced amylase secretion rapidly increases after incubation for about 10 min, as shown previously [16]. The effects of indomethacin on secretion induced by norepinephrine (NE) were similar to those on IPR-induced secretion: that is, it increased the secretion induced by 1 μM NE and inhibited that induced by 100 μM NE (Fig. 3). Indomethacin itself did not affect the spontaneous secretion (Fig. 1) or induce responses by low concentrations of IPR (0.01 μM) and NE (0.1 μM) that alone did not stimulate secretion effectively (Figs 2 and 3). Therefore, PG seems to influence only evoked secretion. Next we examined whether PGs counteracted the effects of indomethacin described above. PGE_2 at 29 nM significantly counteracted the stimulatory and inhibitory effects of indomethacin on the secretions induced by 0.1 and 1.0 μM IPR respectively (Fig. 4). These results suggest important roles of PGs in the amylase secretory response of rat parotid tissue. But PGE_2 alone did not have any significant effect on the spontaneous secretion (not shown) or on the secretion induced by 0.1 or 1.0 μM IPR: the secretory responses induced by incubation for 10 min with 0.1 μM IPR in the absence and presence of indomethacin were 180.2 ± 17.0 and 185.7 ± 13.8 mg maltose/100 mg tissue (N = 9), respectively, and those induced by 1.0 μM IPR with and without indomethacin were 296.2 ± 18.9 and 299.6 ± 26.0 (N = 9), respectively. It seems likely that sufficient PGE_2 for amylase secretion was present in the parotid tissues in the present experimental conditions.

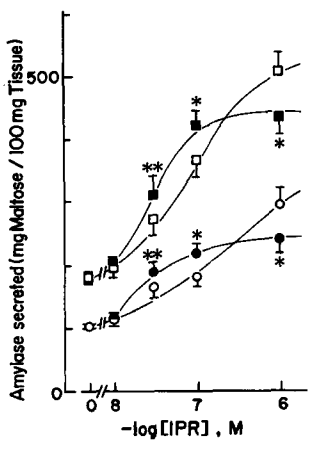

Fig. 2. Effects of indomethacin on amylase secretion induced by IPR. Tissues were incubated for 10 min (○) or 20 min (□) with the indicated concentrations of IPR in the absence (open symbols) and presence (closed symbols) of 14 μM indomethacin. Points and bars are means and standard errors for 5–12 experiments. Significantly different from the value in the absence of indomethacin by Student's paired *t*-test, $^*P < 0.01$ and $^{**}P < 0.02$.

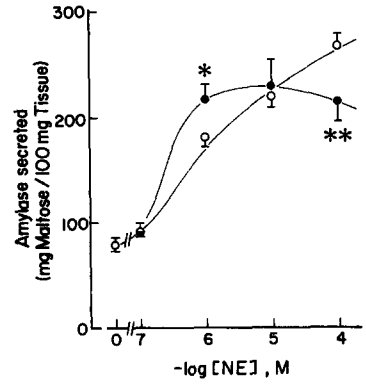

Fig. 3. Effects of indomethacin on amylase secretion induced by NE. Tissues were incubated for 10 min with the indicated concentrations of NE in the absence (○) and presence (●) of 14 μM indomethacin. Points and bars are means and standard errors for 5–8 experiments. Significantly different from the value in the absence of indomethacin by Student's paired *t*-test, $^*P < 0.02$ and $^{**}P < 0.05$.

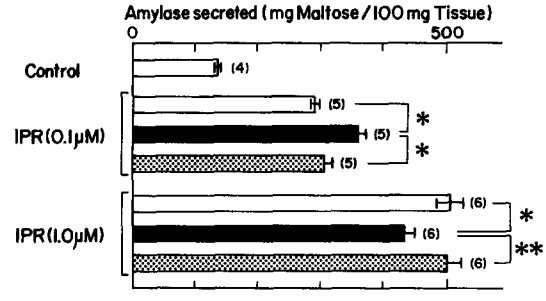

Fig. 4. Effects of indomethacin and PGE_2 on amylase secretion induced by IPR. Tissues were incubated for 20 min with the indicated concentrations of IPR in the absence (open columns) and presence (closed columns) of 14 μM indomethacin and of 14 μM indomethacin with 29 nM PGE_2 (dotted columns). Columns and bars are means and standard errors; the numbers of experiments are shown in parentheses. Significantly different from the value in the presence of indomethacin by Student's paired *t*-test, $^*P < 0.01$ and $^{**}P < 0.02$.

The importance of cyclic AMP in the β-agonist-induced secretory response of the rat parotid tissue has been widely accepted [18–21], although some recent findings are incompatible with this idea [22–26]. It is also well known that PGs elevate the intracellular cyclic AMP level in a variety of tissues [27–29]. The present results may indicate an interaction between β-agonist and PGs in the synthesis of cyclic AMP in rat parotid tissue. There are some opposing reports on the role of endogenous PGs in cholinergic neurons. That is, PGs have been suggested to maintain excitability of cholinergic neurons in the myenteric plexus [3, 6]. On the other hand, PGs were found to inhibit ACh release in a sciatic nerve–muscle preparation of the frog [30], the superior cervical ganglion of the guinea pig [31] and the rabbit [32], a phrenic nerve–diaphragm preparation of the rat [33], circular muscle strips of dog trachea [5] and guinea pig phrenic nerve–diaphragm and chicken parasympathetically innervated oesophagus preparations [7]. However, the present study showed two opposite effects of indomethacin in the same tissue, a stimulatory effect and an inhibitory effect, depending on the concentration of β-agonists. These two opposite effects of indomethacin in the rat parotid tissue are unique, though details of the functional role of endogenous PGs are still unknown.

In summary, indomethacin increased the secretion by low concentrations of isoproterenol (IPR, 30–100 nM) and decreased the secretion by a high concentration of IPR (1 μM). Indomethacin also had opposite effects on the secretions induced by low and high concentrations of norepinephrine (NE). PGE_2 did not affect the secretions induced by IPR and NE, but it reversed the stimulatory and inhibitory effects of indomethacin. These findings suggest that PG has a role in modulating the amylase secretory response of the rat parotid gland.

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Biochemical Pharmacology, Vol. 40, No. 2, pp. 393–397, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Induction of hepatic microsomal P450 I and IIB proteins by hyperketonaemia

(*Received* 21 *December* 1989; *accepted* 16 *March* 1990)

Since the initial publication of Dixon *et al.* [1], a plethora of studies have demonstrated unequivocally that chemically-induced type I, insulin-dependent diabetes modulates the microsomal metabolism of various model substrates [2, 3], and similar findings have been reported in spontaneously diabetic rats [4]. The above studies did not address the selective effect of diabetes on specific cytochrome P450 proteins. We have recently demonstrated that in streptozotocin (STZ)-induced diabetes the O-dealkylations of ethoxyresorufin and pentoxyresorufin, two substrates routinely used to monitor P450 I and P450 IIB activity, respectively [5, 6], were markedly elevated and successfully antagonized by insulin therapy [7]. Moreover, rats rendered hyperketonaemic by the daily administration of triacylglycerols also displayed high dealkylase activities when compared to control animals, and this observation led us to conclude that the ketone bodies may mediate, at least partly, the diabetes-induced increases in these two activities [8].

The use of diagnostic substrates in detecting changes in the levels of specific families of cytochrome P450 suffers from three major disadvantages. Firstly, they do not allow the distinction between proteins belonging to the same family/subfamily and secondly the possibility that an as yet uncharacterized form of microsomal cytochrome P450 catalysing to some extent the diagnostic substrates is present, cannot be excluded. Finally, the modulating agent may still be present in the microsomes employed in the assays causing inhibition and consequently underestimation of the degree of induction. However, this last possibility is extremely unlikely to contribute to the observed effects of STZ as the half-life of this drug is less than 15 min. In order to overcome the first two difficulties, we investigated the effect of streptozotocin-induced diabetes and the diet-induced hyperketonaemia on the hepatic microsomal levels of P450 I and P450 IIB proteins determined immunologically using monospecific antibodies.

Materials and Methods

Medium chain triacylglycerols (Cow and Gate Ltd, Trowbridge, U.K.), long-acting monocomponent human insulin (Ultratard, Novo Industries, Copenhagen, Denmark), nicotinamide (Sigma Chemical Co., Poole, U.K.) and peroxidase-linked donkey anti-sheep IgG and peroxidase-linked donkey anti-rabbit IgG (Guildhay Antisera, Guildford, U.K.) were all purchased. The preparation of medium chain triacylglycerols used comprised fractionated coconut oil predominantly composed of the triacylglycerols of octanoic and decanoic acids. The purification and characterization of cytochrome P450 IA1 and the production of antibodies recognizing both A1 and A2 proteins have already been described [9]. Anti-cytochrome P450 IIB, recognizing both B1 and B2 proteins was a generous gift from Dr C. R. Wolf, Imperial Cancer Research Fund, Hugh Robson Building, George Square, Edinburgh, U.K.

Male Wistar albino rats (Experimental Biology Unit, University of Surrey) weighing 180–200 g were used in two experimental studies. In the first study, animals were randomly divided into four groups each comprising four animals. One group served as control, the second group received a single intraperitoneal administration of STZ (65 mg/kg) dissolved in 0.5 M citrate buffer (pH 4.5); the third group received, in addition to STZ, two intraperitoneal doses of nicotinamide (350 mg/kg), one 10 min prior to, and the other 3 hr after the administration of STZ (STZ + nic); finally the fourth group received, in addition to STZ, single daily increasing doses of insulin (STZ + ins) as previously described [7]. All animals were killed 22 days after commencement of treatment and 24 hr after the last administration of insulin. In the second study, rats were randomly divided into three groups each comprising four animals. One group served as control, the second group received a single dose of STZ as described above, while the third group received daily intragastric intubation of medium chain triacylglycerols (MCT) (8 g/kg). These animals were also killed 22 days after the commencement of the treatment. Lastly, to serve as positive controls for the induction of the P450 I and P450 IIB families, groups of previously untreated rats were treated with single daily intraperitoneal administrations of either 3-methylcholanthrene (3MC, 25 mg/kg) or phenobarbitone (PB, 80 mg/kg), respectively, for 3 days, all animals being killed 24 hr after the last injection. In all cases, livers were immediately excised and hepatic microsomal fractions prepared as previously