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Adrenoceptors in the cat choledocho-duodenal junction studied *in situ*

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Summary

1. The effects of adrenaline, noradrenaline, isoprenaline and terbutaline (PINN), 1-(3,5-dihydroxyphenyl)-2(*t*-butylamino)-ethanol, on the sphincter of Oddi were studied in anaesthetized cats. Both adrenaline and noradrenaline (1–4 μg/kg) increased resistance to flow through the sphincter. This effect was blocked by dibenamine (0·5–2·5 mg/kg) and by phenoxybenzamine (0·5–1·0 mg/kg).

2. Isoprenaline (0·5–5·0 μg/kg) as well as terbutaline (0·5–10·0 μg/kg) inhibited the spontaneous sphincter activity and decreased flow resistance. In these doses, isoprenaline provoked marked tachycardia and relaxation of the duodenum, whereas the cardial and intestinal effects of terbutaline were minimal. The effects were blocked by propranolol (0·5–1·0 mg/kg).

3. The results confirm earlier observations of the presence of α-adrenoceptors active in contraction of the sphincter musculature and of β-adrenoceptors active in its relaxation. It is suggested that the β-adrenoceptors belong to the β_2 type according to Lands' classification.

Introduction

Many studies have been devoted to determining the action of sympathomimetic agents on the choledochoduodenal junction (for references see Benzi, Berté, Crema & Frigo, 1964). It now seems possible to explain earlier contradictory results by the existence of excitatory α-adrenoceptors and inhibitory β-adrenoceptors in the smooth musculature of the choledochoduodenal junction. Crema and co-workers investigated the effects of sympathomimetic amines on the sphincter of Oddi in different species (Benzi & Crema, 1961 ; Benzi *et al.*, 1964 ; Crema & Benzi, 1961 ; Crema, Benzi & Berté, 1962 ; Crema & Berté, 1963 ; Crema, Berté, Benzi & Frigo, 1963 ; Crema, Benzi, Frigo & Berté, 1965). The excitatory effect of α-adrenoceptor stimulation was shown both *in vivo* and *in vitro*. Relaxation due to β-adrenoceptor stimulation was also found but difficulties were experienced in confirming this response of the sphincter *in vitro* (Crema & Benzi, 1961 ; Crema *et al.*, 1962). However, relaxation due to β-adrenoceptor stimulation has been found in different preparations of the isolated sphincter of Oddi from the cat (C. G. A. Persson, to be published). According to Lands, Groblewski & Brown, 1966 ; Lands, Luduena & Buzzo, 1967) different kinds of β-adrenoceptors can be distinguished: β_1 (active in cardiac acceleration, small intestine relaxation) and β_2 (active in bronchodilatation). It was thought of interest to investigate the nature of the β-adrenoceptor present in the sphincter of Oddi. This work reports the effect of a selective β_2-adrenoceptor

stimulating compound, terbutaline (Bergman, Persson & Wetterlin, 1969) on the cat choledochoduodenal junction, compared with other α- and β-adrenoceptor stimulating agents.

Methods

The method used was a modification of that described by Liedberg & Halabi (1970).

Thirty-four healthy cats of both sexes were used. They were fasted for 24 h before the experiment. Anaesthesia was induced with pentobarbital 40 mg/kg intraperitoneally and maintained with intravenous pentobarbital at a level where the corneal reflexes had disappeared. Midline laparotomy was performed, and the terminal portion of the common bile duct was isolated and catheterized towards the duodenum. The bile ducts above the catheter were drained to prevent distension. The pressure was continuously recorded during constant rate saline perfusion through the choledochoduodenal junction. The perfusion rate was 0·05 ml/min, unless the initial recording of pressure showed less than 10 cm H_2O, when it was increased to 0·2 ml/min. A pressure range of 10–25 cm H_2O in the common duct catheter was thus achieved. When suitable for recording, the pressure tracing was damped by connecting a water manometer (inner diameter 0·2 cm) to the common duct catheter. The duodenal pressure close to the papilla of Vater was continuously recorded by an open tip catheter inserted through a gastrotomy into the duodenum.

The R-deflection in the e.c.g. was used to trigger a tachometer. All recordings were made on Grass polygraph model 7PI.

The drugs were injected intravenously in the vena cava through a catheter introduced through the femoral vein. The following drugs were used: (±)-terbutaline (PINN) sulphate, (±)-isoprenaline hydrochloride, (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, propranolol hydrochloride, dibenamine hydrochloride, phenoxybenzamine hydrochloride, cholecystokinin (Prof. J. E. Jorpes, G.I.H. Laboratories, Karolinska Inst., Stockholm). Solutions were prepared from fresh glass-distilled water before each experiment. In the case of isoprenaline, the solution was stabilized with 0·2 mg/ml ascorbic acid. The amount of each drug is expressed in terms of the base.

Results

The perfusion pressure through the sphincter was not constant but showed rhythmic pressure waves with a frequency of 3–7/minute. These waves often changed pattern during the experiment. They probably reflected changes in the sphincter tonus, as they occurred independently of the duodenal motility.

Noradrenaline and adrenaline (1–4 μg/kg) constantly and reproducibly raised the perfusion pressure in ten of eleven cats (Fig. 1). In one cat, the action of noradrenaline could be shown only after β-adrenoceptor blockade. The rise lasted 0·5–1·5 min and was sometimes followed by decreased passage pressure. The excitatory response to both amines was blocked by phenoxybenzamine 0·5–1·0 mg/kg (five cats) (Fig. 1) or by dibenamine 0·5–2·5 mg/kg (six cats). The β-adrenoreceptor blocking agent propranolol did not diminish the increase in perfusion pressure produced by noradrenaline or adrenaline. Noradrenaline and adrenaline

relaxed the duodenum, and this relaxation was not abolished by the α-adrenoceptor blocking agents. Neither phenoxybenzamine nor dibenamine affected sphincter or intestinal spontaneous activity.

Terbutaline and isoprenaline depressed the sphincter activity as shown by the disappearance of the rhythmic pressure waves and decreased the flow resistance at the choledochoduodenal junction (Figs. 2, 3 and 4). The duration of action was 2–6 min and was not always dose-dependent. The duration of the effects on the sphincter and the intestine was not uniform (Fig. 4). Terbutaline exhibited its response in the dose range 0·5–5·0 μg/kg on thirty cats and in the dose range 4–10 μg/kg on four cats. In all thirty-four experiments, isoprenaline produced its effect in doses 0·5–5·0 μg/kg. The response to these β-adrenoceptor stimulating agents varied during the experiments and were not related to variations in the perfusion pressure. Doses of isoprenaline equipotent with terbutaline in their action on the sphincter, decreased duodenal pressure more markedly than terbutaline and the effect of isoprenaline on heart rate was much more pronounced than that of terbutaline (Figs. 2, 3 and 4). Tachycardia was consistent throughout the experiments. On rare occasions, there was an initial rise in intestinal and sphincter tonus after injection of terbutaline or isoprenaline. The excitatory effect of isoprenaline on intestine has been described earlier (Ahlquist, 1967).

Eight cats, showing a constant response in sphincter tonus to terbutaline and isoprenaline, were treated with propranolol (0·5–1·0 mg/kg). Propranolol by itself tended to cause a slight and transient increase in the perfusion pressure. It nullified all the effects of terbutaline and isoprenaline (Fig. 4). Dibenzyline and dibenamine did not seem to affect the response to terbutaline and isoprenaline.

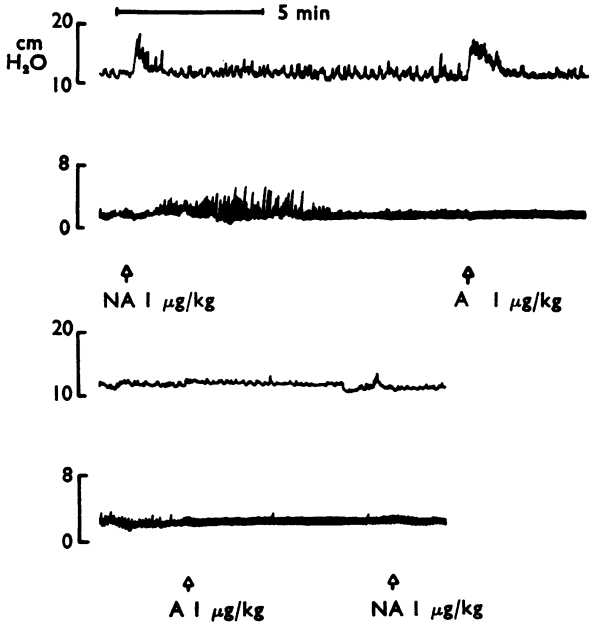

FIG. 1. Effects of noradrenaline (NA) and adrenaline (A) on sphincter (upper record) and duodenum (lower record) before and after α-adrenoceptor blockade with dibenzyline 1·5 mg/kg given 15–20 min before A and NA (the lower two records).

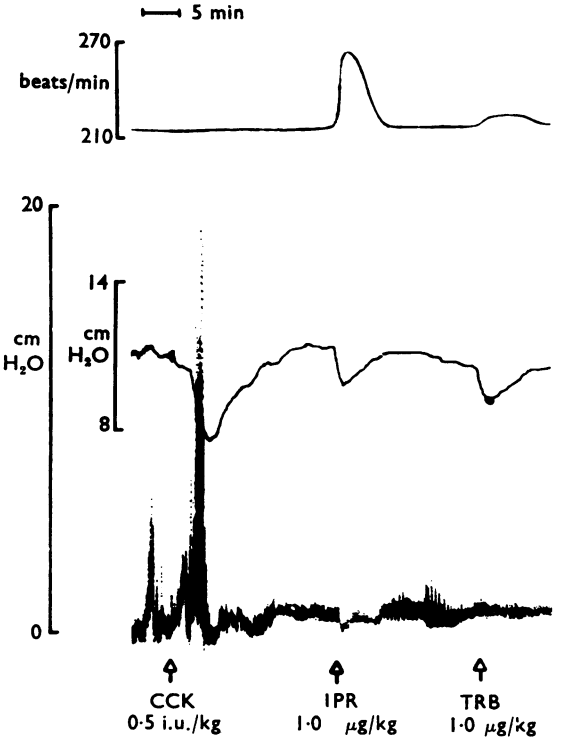

FIG. 2. Effect of cholecystokinin (CCK), isoprenaline (IPR) and terbutaline (TRB) on heart rate, sphincter resistance, and duodenal pressure. Upper record, heart rate (beats/min); middle record, sphincter resistance (cm H_2O); lower record, duodenal pressure (cm H_2O).

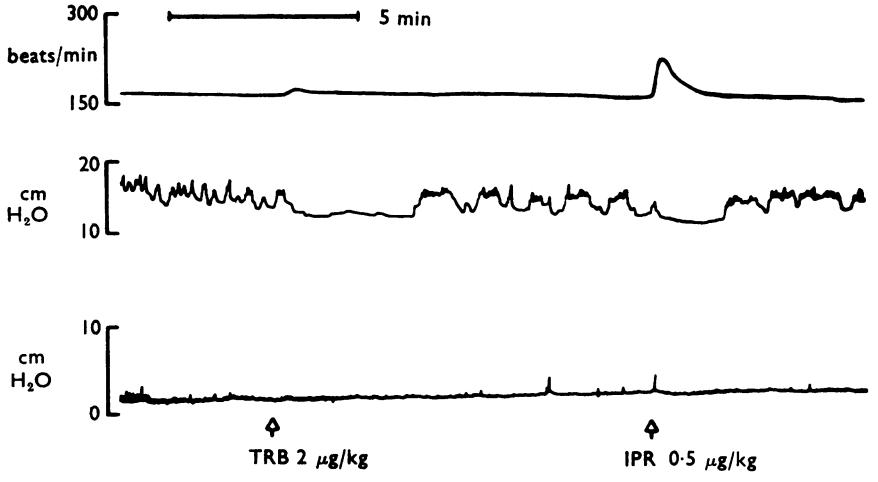

FIG. 3. Records as in Fig. 2. Effect of isoprenaline (IPR) and terbutaline (TBR) on spontaneous sphincter activity (undamped curve).

Cholecystokinin (CCK), 1–3 i.u./kg, increased the intestinal activity and relaxed the sphincter (fifteen cats) (Fig. 2). Its effect was not changed by β-adrenoceptor blockade with propranolol (three cats).

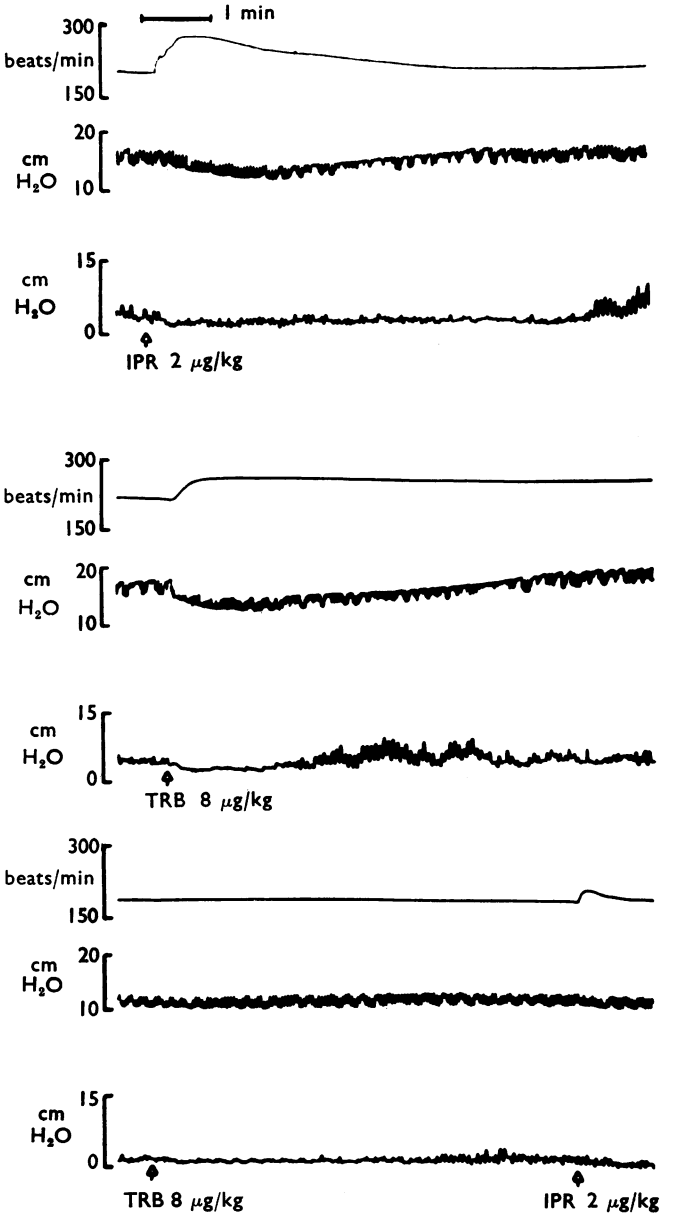

FIG. 4. Records as in Fig. 2. Effects of isoprenaline (IPR) and terbutaline (TBR) on heart rate, sphincter resistance, and duodenal motility before and after propranolol. Propranolol 1 mg/kg was given 15–20 min before TBR and IPR (the three lower curves).

Discussion

For anatomical reasons (see, for example, Boyden, 1957), it could be difficult to evaluate effects on the sphincter of Oddi *in situ* without the disturbing influence of the surrounding smooth musculature of the duodenum. However, earlier workers have presented strong evidence in favour of the existence of an independent sphincter mechanism (Bergh & Layne, 1940 ; Lueth, 1931 ; Jacobsson, Lanner & Rådberg, 1957 ; Hauge & Mark, 1965 ; Wyatt, 1967 ; Liedberg & Halabi, 1970). This concept is further strengthened by the present investigation. Thus α-adrenoceptor stimulating agents cause a rise in perfusion pressure, while a relaxation of the duodenum is recorded ; cholecystokinin relaxes the sphincter but stimulates duodenal motility, and by selective β-adrenoceptor stimulation, a relaxation of the sphincter can be achieved without any recorded effect on the intestine. Furthermore, the rhythmic pressure waves recorded during the perfusion of the sphincter were not related to duodenal or respiratory activity.

The anaesthetic agent pentobarbital perhaps reduces smooth muscle tonus in the sphincter of Oddi and thus diminishes the possibility of relaxing agents affecting the sphincter. Thus, in some experiments, additional anaesthetic doses of pentobarbital 6 mg/kg intravenously depressed the spontaneous activity of the sphincter. Induction of anaesthesia with α-chloralose 60 mg/kg intraperitoneally did not seem to increase the sensitivity to the β-adrenoceptor stimulating agents, and the chloralose-treated cats also showed depressed sphincter activity when given small additional doses of pentobarbital.

The rise in passage pressure through the sphincter caused by noradrenaline and adrenaline is probably mediated through α-adrenoceptor stimulation, as the effect was blocked by phenoxybenzamine or dibenamine (Fig. 1). The excitation of α-adrenoceptors agrees well with the findings of Crema *et al.* (1965) on the guinea-pig sphincter of Oddi, but is not in complete agreement with the results of Benzi *et al.* (1964) on the effect of α-adrenoceptor stimulation on the flow through the choledochoduodenal junction of the cat and the dog. They most frequently found an increase in flow after noradrenaline and adrenaline. However, they also found an initial decrease in flow followed by an increase. Erdmann & Henne (1953) recorded an increase in sphincter tonus in the cat after the administration of noradrenaline and adrenaline. Dardik, Schein, Warren & Gliedman (1969) found a mixed response to noradrenaline on passage pressure in the sphincter of Oddi in dogs. This inconsistent response to α-adrenoceptor stimulation of the sphincter of Oddi is possibly due to the α-adrenoceptor stimulating agents relaxing the duodenum. Perhaps, depending on the methods used, this effect might in some cases override the response of the smooth musculature at the choledochoduodenal junction to excitation of α-adrenoceptors. On some occasions in our experiments the initial rise in perfusion pressure was followed by a depressed sphincter activity. This observation could be explained as a biphasic response due to the mixed α- and β-adrenoceptor stimulating properties of the compounds.

The reports on the effect of the β-adrenoceptor stimulating agent isoprenaline on the sphincter of Oddi are unanimous. An increase in flow through the sphincter and a decrease in passage pressure are consistently shown (Benzi *et al.*, 1964 ; Crema *et al.*, 1965 ; Dardik *et al.*, 1969). However, it has not been determined to what degree the inhibitory action of isoprenaline on the duodenum has contributed to

the effect on the sphincter. This possibility must be considered, especially when isoprenaline is given in large doses such as 30 μg/kg (Dardik *et al.*, 1969). Our observations on isoprenaline and terbutaline support the earlier observations that β-adrenoceptor stimulation relaxes the sphincter. Terbutaline is a selective β-adrenoceptor stimulating agent (Bergman *et al.*, 1969). Its pharmacology was evaluated by Persson & Olsson (1970) and Persson & Johnson (1970), who showed terbutaline to act selectively on the β_2-adrenoceptors (β_1 and β_2 according to the classification of Lands *et al.*, 1966, 1967). In all cats studied, terbutaline and also isoprenaline unequivocally decreased the resistance to flow through the sphincter, although to a varying degree. In eight cats used to test the effect of β-adrenoceptor blockade, propranolol consistently blocked the actions of low doses of terbutaline and isoprenaline, supporting the view that these agents stimulate β-adrenoceptors in the sphincter.

The relaxation of the sphincter produced by CCK was not affected by β-adrenoceptor blockade with propranolol (three cats). This finding supports the conclusion of Hedner & Rorsman (1969) that the action of CCK on the sphincter of Oddi does not depend on receptors or mechanisms belonging to the autonomic nervous system. The transient increase in passage pressure after propranolol, which occurred in some animals, agrees with the observations of Dardik *et al.* (1969).

The sphincter of Oddi did not show a stable sensitivity in its response to isoprenaline or terbutaline. This variation was not obviously related to changes in the recorded perfusion pressures through the sphincter or in intestinal pressure and made evaluation of meaningful dose response relations impossible. A varied degree of response to one dose was also recorded with CCK, which was used to test the sphincter for relaxant properties in fifteen cats.

A dose of terbutaline that relaxed the sphincter had markedly less effect on the duodenal pressure and the heart frequency than isoprenaline had in doses with an equal effect on the sphincter. A difference in duration of action, especially for terbutaline on sphincter and intestine (the action on the intestine being of shorter duration) (Fig. 4), supports our view that terbutaline, compared with isoprenaline, is relatively more active on the sphincter than on the intestine.

The results obtained with the two different β-adrenoceptor stimulating agents, isoprenaline and terbutaline, on the sphincter of Oddi in cats do not permit definite conclusions about the type of β-adrenoceptor present in the sphincter, but indicate that they are related more to the β_2-type of bronchial smooth musculature than to the β_1-type found in the heart and the intestine.

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