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INHIBITORY INNERVATION OF CAT SPHINCTER OF ODDI

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1 Electrical stimulation with trains of 0.1–0.2 ms pulses of the cat isolated sphincter of Oddi inhibited the spontaneous contractile activity and lowered base-line tension considerably. A contraction usually followed the period of stimulation.

2 These inhibitory effects were prevented by tetrodotoxin 0.1–0.5 μg/ml but were not reduced by hexamethonium, morphine, or blockade of α- or β-adrenoceptors or cholinoceptors with phenoxybenzamine propranolol or atropine, respectively.

3 Adenosine-5′-triphosphate (ATP) and adenosine-5′-diphosphate (ADP) inhibited the spontaneous sphincter activity and caused relaxation thus mimicking the effects of the C-terminal octapeptide of cholecystokinin (C8-CCK), isoprenaline and prostaglandins E_1 and E_2.

4 ATP alone (> 100 μg/ml) or ATP (> 10 μg/ml) plus dipyridamole (1 μg/ml), relaxed the sphincter to the same degree as did the field stimulation.

5 In sphincters maximally contracted by acetylcholine, the effect of stimulation was more marked than that recorded in uncontracted preparations.

6 The present findings suggest that the sphincter of Oddi receives inhibitory nerves that are neither cholinergic nor adrenergic.

Introduction

The activity of the sphincter of Oddi might be controlled by several mechanisms (Hallenbeck, 1967; Persson, 1973; Andersson, Andersson, Hedner & Persson, 1973). The most important is considered to be the hormone cholecystokinin, which inhibits sphincter activity and tone without involving nerves (Persson, 1973). The role of the nerves is not settled. In the cat sphincter of Oddi the adrenergic innervation may counteract the effect of cholecystokinin, as stimulation of adrenergic nerves and administration of the transmitter amines contract the sphincter and increase its activity (Persson, 1973). Less is known about the cholinergic control, but exogenous acetylcholine contracts the sphincter of Oddi in most species (Hallenbeck, 1967).

Studies of gastro-intestinal motility have repeatedly demonstrated the presence of non-adrenergic inhibitory and non-cholinergic excitatory nerves (Bortoff, 1972) and the non-adrenergic nerves may be purinergic (see review by Burnstock, 1972).

The sphincter of Oddi may receive an innervation that is neither cholinergic nor adrenergic (Persson, 1971b), a suggestion which is further supported by the present experiments.

Methods

Fourteen adult cats, fasted for 24 h but allowed water, were anaesthetized with sodium pentobarbitone (Abbott) (40 mg/kg i.p.) and bled out. The sphincter of Oddi was dissected free from surrounding duodenum and the bile duct was cut off. Without occluding the orifices, the sphincter was mounted as a longitudinal preparation in an organ bath, one end being connected to a strain-gauge transducer (Persson, 1971a). The bath contained Krebs solution with the following composition (mM): NaCl 118.0, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, $NaHCO_3$ 24.9, KH_2PO_4 1.15 and glucose 5.5; pH was 7.4. It was kept at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2. Changes in isometric longitudinal tension of the sphincter were recorded on a Grass Polygraph by means of strain-gauge transducers (FTO3).

Two platinum electrodes connected to an electronic stimulator (Grass S48 Stimulator) were fixed vertically, approximately 5 mm apart on opposite sides of the sphincter preparation. The preparation was stimulated at supra-maximal voltage (40 V) with trains of rectangular pulses delivered at regular intervals (usually 100 seconds). After preliminary trials at 0.05–0.5 ms, the pulse width was fixed at 0.1 ms and 5 Hz for most experiments.

The drugs used were: acetylcholine chloride (Calbiochem, U.S.A.), adenosine-5′-diphosphate (disodium salt) (Sigma Chemical Company, U.S.A.), adenosine-5′-triphosphate (disodium salt) (Sigma Chemical Company, U.S.A.), C-terminal octapeptide of cholecystokinin (C8-CCK) (Squibb, U.S.A.), dipyridamole (Boehringer Ingelheim, Germany), hexamethonium bromide (May & Baker Ltd.), (±)-isoprenaline chloride (Sigma Chemical Company, U.S.A.), morphine chloride (Pharmacopoea Nordica) (morphine hydrochloride), (−)-noradrenaline bitartrate (Sigma Chemical Company, U.S.A.), phenoxybenzamine hydrochloride (Smith, Kline & French), propranolol hydrochloride (ICI Ltd.), tetrodotoxin (Sankyo, Japan). The drugs were dissolved in fresh glass-distilled water before each experiment. Solutions of isoprenaline and noradrenaline contained ascorbic acid (0.2 mg/ml).

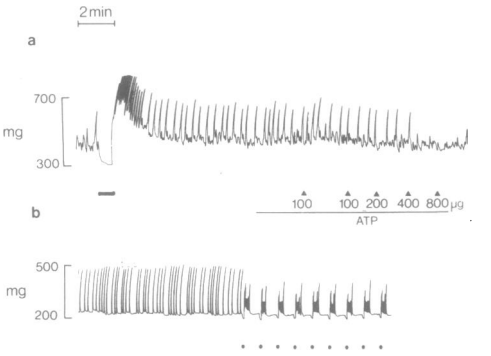

Figure 1 (a) Effect of electrical stimulation and adenosine triphosphate (ATP) on spontaneous contractions of the sphincter of Oddi. At bar, electrical stimulation for 40 s inhibited contractile activity. A marked excitation occurred after the stimulus ended. ATP added cumulatively to the bath (50 ml) inhibited the spontaneous activity. (b) Effect of electrical stimulation (dots) at regular intervals on spontaneous activity of a sphincter of Oddi. Sphincter tone was reduced; note also changes in the pattern of contractile activity.

Results

The sphincters exhibited spontaneous rhythmic contractile activity. Electrical stimulation inhibited the contractile activity and, except in two preparations, greatly lowered the base-line tension (Figures 1, 2, 4 and 5). In two preparations the response to stimulation was recorded as only an inhibition of the rhythmic activity. In seven preparations the period of stimulation was followed by contraction (Figure 1). The pulses were of 0.1 or 0.2 ms duration. Above 0.3 ms some of the response remained after tetrodotoxin (0.1–0.5 µg/ml) had completely inhibited the response to 0.2 ms pulses. Tetrodotoxin (TTX) (0.5 µg/ml) was fully effective 5 min after its addition to the bath, preventing the inhibitory effects and post-stimulus excitation of the sphincter. After washout of TTX, the response to electrical stimulation gradually recovered (Figure 2). As reported previously (Persson, 1971b), TTX by itself, in some preparations, produced a stimulant effect which showed tachyphylaxis.

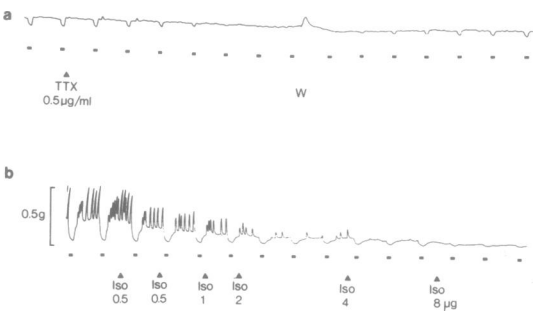

Figure 2 (a) Tetrodotoxin (TTX) inhibited the effect of field stimulation (bars) in cat isolated sphincter of Oddi without spontaneous contractile activity. After washing (W), the relaxant effect gradually returned. Due to tachyphylaxis no excitatory effect of TTX can be seen. (b) Isoprenaline (ISO) added cumulatively to the bath (50 ml) relaxed the sphincter; high concentrations relaxed the sphincter to the same extent as with field stimulation (bars).

The inhibitory response to electrical stimulation was not changed by atropine (0.1 µg/ml), phenoxybenzamine (0.1 µg/ml) or propranolol (0.1 µg/ml); the antagonists were used in concentrations which prevented, respectively, contractions induced by acetylcholine (0.1 µg/ml) and noradrenaline (0.1 µg/ml) or relaxations due to isoprenaline (0.2 µg/ml). Hexamethonium (100 µg/ml) was also without effect, as was morphine (0.1–10 µg/ml); a range of frequencies between 1 and 5 Hz was tried in the presence of morphine. The blocking agents were left in contact with the sphincter preparations for up to 20 minutes.

ATP (2–30 µg/ml) or adenosine-5′-diphosphate (ADP, 4–30 µg/ml) inhibited spontaneous activity of the sphincter but the basal tension was only slightly

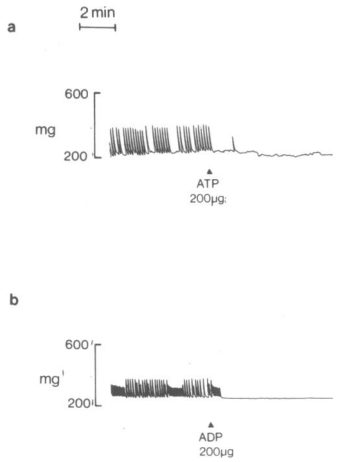

Figure 3 (a) Inhibition of contractile activity in the sphincter of Oddi by adenosine triphosphate (ATP). (b) Inhibitory effect of ATP mimicked by ADP. Bath volume, 50 ml.

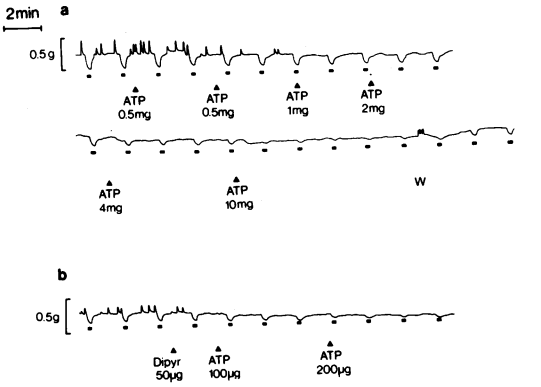

Figure 4 (a) Adenosine triphosphate (ATP) added cumulatively to the bath gradually relaxed the isolated sphincter of Oddi to the same extent as field stimulation (bars). (b) Potentiation of effect of ATP by dipyridamole (Dipyr.). Electrical stimulation at bars. Bath volume, 50 ml.

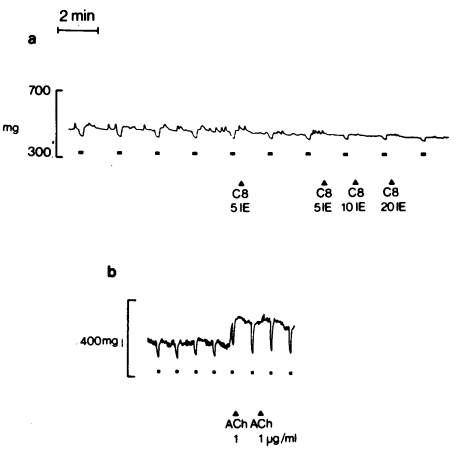

Figure 5 (a) Reduction in tone of isolated sphincter of Oddi and response to electrical stimulation by octapeptide of cholecystokinin (C8); IE = Ivy dog units of cholecystokinin. Bath volume, 50 ml. (b) Pronounced lowering of sphincter tone by electric stimulation (bars). When the sphincter was maximally contracted by acetylcholine (ACh) the relaxation was even more marked.

lowered (Figures 3 and 4). In concentrations above 100 µg/ml, ATP produced a relaxation as pronounced as that induced by electrical stimulation (Figure 4). In the presence of dipyridamole, 1 µg/ml, a pronounced relaxation was seen at lower concentrations of ATP (approx. 10 µg/ml) (Figure 4); dipyridamole alone did not change the response to field stimulation. Prostaglandins E_1 and E_2, C8-CCK and isoprenaline also inhibited the contractile activity, and lowered the base-line tension (cf. Persson, 1973; Andersson *et al.*, 1973), producing a maximum equalled by that obtained with field stimulation (Figures 2 and 5).

When the sphincter was maximally contracted by acetylcholine (1 µg/ml), the effect of electrical stimulation was more marked than that recorded in uncontracted preparations (Figure 5). In the presence of ACh (1 µg/ml) ATP at a given concentration relaxed the sphincters less than it did when they were uncontracted. In addition, ATP up to 1 mg/ml in contracted preparations did not produce a relaxation as pronounced as that induced by electrical stimulation. The post-stimulation excitatory responses recorded in seven of the fourteen sphincters (Figure 1) were not inhibited by hexamethonium (100 µg/ml) or indomethacin (1–4 µg/ml), and they were only slightly diminished by atropine (0.1 µg/ml) or phenoxybenzamine (0.1 µg/ml). The size of the post-stimulus response gradually diminished during the experiment and in five preparations had vanished after approximately 3 h while the inhibitory effect remained intact. Some preparations lost spontaneous activity after 1–3 h and an inhibitory effect was seen only as a lowering of base-line tension (Figure 2).

Discussion

Responses of the isolated sphincter of Oddi preparation agree well with those obtained *in vivo* and the changes in longitudinal tension (oblique and longitudinal muscle fibres) closely reflect changes in resistance to flow through the sphincter (Persson, 1973). Resistance of the sphincter is influenced by the intrinsic nerves, which are perhaps predominantly

inhibitory. Although high concentrations were required to produce a relaxation as great as that induced with electrical stimulation, ATP and ADP were found to relax the sphincter, a finding which suggests that 'purinergic' nerves may be involved (see Burnstock, 1972). It has been shown that ATP alone does not mimick the neural response but does so in the presence of dipyridamole (Coleman & Levy, 1974). In the present study, dipyridamole diminished the concentration of ATP needed to produce inhibitory effects; this potentiation may have been due to the uptake of adenosine being blocked by dipyridamole.

Sphincter relaxation was also produced by isoprenaline, acting on β-adrenoceptors, by the octapeptide of cholecystokinin, and by prostaglandins E_1 and E_2. These effects have also been shown previously (Persson, 1971a; 1973; Andersson *et al.*, 1973). However, since the effects of cholecystokinin, prostaglandins, ATP and ADP cannot be selectively antagonized, their possible participation in the inhibitory effect of field stimulation is difficult to investigate.

A cholinergic or adrenergic component of post-stimulus contractions cannot be excluded nor assessed quantitatively due to the development of tachphylaxis. The contractions may have been myogenic in origin, as suggested by Furness (1971) for the guinea-pig intestine but this is unlikely since they were prevented by TTX. On the other hand, the phenomenon might be due to increased prostaglandin synthesis as recently reported for intestinal muscle by Burnstock, Cocks, Paddle & Staszewska-Barczak (1975). This explanation is also not plausible since prostaglandins E_1, E_2 and $F_{2\alpha}$ all inhibit the isolated sphincter of Oddi (Andersson *et al.*, 1973).

Persson (1971b) found that TTX apparently stimulated the isolated sphincter of Oddi; this did not seem to be due to inhibition of cholinergic or adrenergic nerves. This effect of TTX, which was confirmed in the present experiments, may result from inhibition of conduction in the non-adrenergic, possibly 'purinergic', inhibitory nerves.

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