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Effects of pinaverium bromide and verapamil on the motility of the rat isolated colon

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1 Pinaverium bromide was 30 times less potent than verapamil in inhibiting intraluminal pressure responses of *in vitro* rat colonic segments to barium chloride, acetylcholine, FK 33-824 or field stimulation.

2 The inhibitory effects of both verapamil and pinaverium bromide on the pressure responses to field stimulation were antagonized similarly by exogenous calcium administration.

3 These results support the concept that pinaverium bromide acts on calcium channels in the smooth muscle cell membrane.

Introduction

Pinaverium bromide is a spasmolytic drug with no anticholinergic effect (Bretaudeau *et al.*, 1975). In recent years, this compound has been recommended for the treatment of functional colonopathies. Several clinical studies, three of which were double blind (Dubarry & Quinton, 1977; Levy *et al.*, 1977; Delmont, 1981) point to a significant effect of this compound in irritable colon. However, little experimental work has been carried out on the mode of action of pinaverium on the colon.

Itoh & Takahashi, (1981) showed that in pinaverium-treated conscious dogs the contractile activity of the stomach, small intestine and colon was reduced. Also, they observed that the progression of the myoelectric complex along the gastrointestinal tract was slowed down. This has been confirmed by Grenier *et al.* (1983) using the small intestine of dogs. Droogmans *et al.* (1983) investigated the effects of pinaverium on electrical and mechanical activities and on ^{45}Ca exchange in the guinea-pig taenia coli and ileum. They found that pinaverium bromide 10^{-6}–10^{-5}M induced a spasmolytic effect on the longitudinal muscle layer of the colon and showed that the calcium influx in the muscle cells was reduced in a manner comparable to that seen with D-600 (methoxyverapamil), a typical antagonist of voltage-dependent calcium channels. They concluded that pinaverium bromide affects the voltage-dependent channels more than the hormone-sensitive channels.

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Whereas Droogmans *et al.* (1983) used longitudinal smooth muscle strips of guinea-pig caecum, the present studies investigated the actions of pinaverium on the rat isolated colon, the smooth muscle layer of which is constituted mainly of circular muscle. The latter probably play a role in spastic diseases of the colon.

Methods

Male Wistar rats weighing 120 ± 20 (s.e.mean) g were used. After an overnight fast the colon was removed under ether anaesthesia, flushed with oxycarbonated Tyrode-Ringer solution and divided into three equally sized parts. These colonic segments were subsequently placed in Tyrode-Ringer solution (pH 7.4, composition in mM: NaCl 137, KCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, $NaHCO_3$ 11.9, $NaH_2PO_4.2H_2O$ 0.4 and glucose 5, gassed with 5% CO_2 in O_2) maintained at 37°C. A roller pump provided a continuous replenishment of the bathing fluid in the organ bath whilst a suction pump kept the bath level constant at 40 ml. Intraluminal pressure changes were measured simultaneously in the three colonic segments using a perfusion manometric system via volume displacement transducers (Statham P 23b), Philips electromanometers (XM 5101) and recorded by a Cardiopan 8 R multichannel writer (Liechti, Switzerland) (Figure 1).

A drug-induced increase in muscle tone is expressed by the area under the pressure-time curve during the first 5 min of muscle stimulation and in the text is

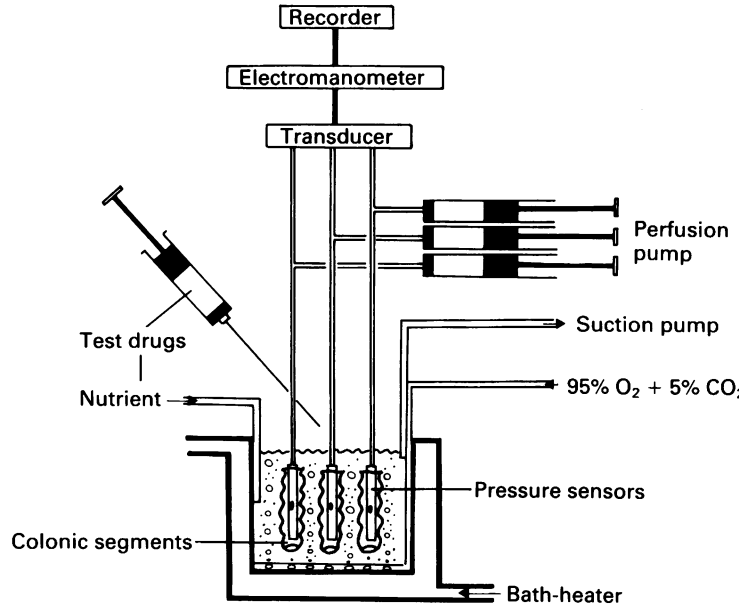

Figure 1 Experimental organ bath set-up for simultaneous intraluminal pressure measurement from three colonic segments.

referred to as the integrated tonic colonic pressure response (ICPR), given in arbitrary units. In the case of field stimulation the peak amplitude of the pressure change is used as the measure of the pressure response.

The test substances were added to the organ bath either with a syringe in a volume not exceeding 0.2 ml or given as a slow perfusion together with the Ringer solution.

The washout of drugs occurred by replenishing the organ bath with fresh Tyrode-Ringer solution within 2 min and subsequently maintaining the flow rate of fresh Ringer solution at 20 ml min^{-1} for at least 20 min.

The colonic tone of the tissue preparations was stimulated by 10^{-3}M barium chloride, 10^{-4}M acetylcholine chloride or 10^{-6}M FK 33-824 or by electrical field stimulation in a random sequence.

Verapamil and pinaverium are difficult to wash out, and the sequence of doses for these test substances could not be randomized, because a small dose always had to be tested before a higher dose so as not to kill too many animals.

Field stimulation using rectangular impulses of 5 mA, 10 ms duration at 10 Hz was elicited via a point electrode in the colonic lumen and a circular electrode placed outside the tissue preparation. This field stimulation induced smooth muscle contractions by a neural pathway since responses were completely blocked by 10^{-6}M tetrodotoxin.

The data were analysed for statistical significance by paired and unpaired Student's *t* test, linear regression analysis, 3 × 3 dose parallel line assay and 2 × 2 dose parallel assay (Colquhoun, 1971; Sachs, 1972).

Drugs

[D-Ala^2, MePhe^4, Met(0)-01^5], a synthetic [Met]enkephalin analogue coded FK33-824 (Sandoz Ltd, Basle, Switzerland), acetylcholine chloride (Baeschlin, Winterthur, Switzerland), $BaCl_2$ (Pharmacopoea Helvetica), verapamil (Orion Pharm., Helsinki, Finland), pinaverium bromide (Kali-Duphar Pharma AG, Berne, Switzerland), tetrodotoxin (Feinbiochemica, Serva, Heidelberg, FRG).

Results

The intraluminal pressure pattern of proximal colonic segments before and after electrical and different chemical stimulation is illustrated in Figure 2. Acetylcholine and barium chloride increased the strength of rhythmic and tonic contractions in the colonic segments without affecting the frequency of rhythmic contractions. FK 33-824 additionally increased the frequency of rhythmic colonic contractions. Electrical field stimulation induced short lasting contractions of high amplitude. Comparable changes in the motility

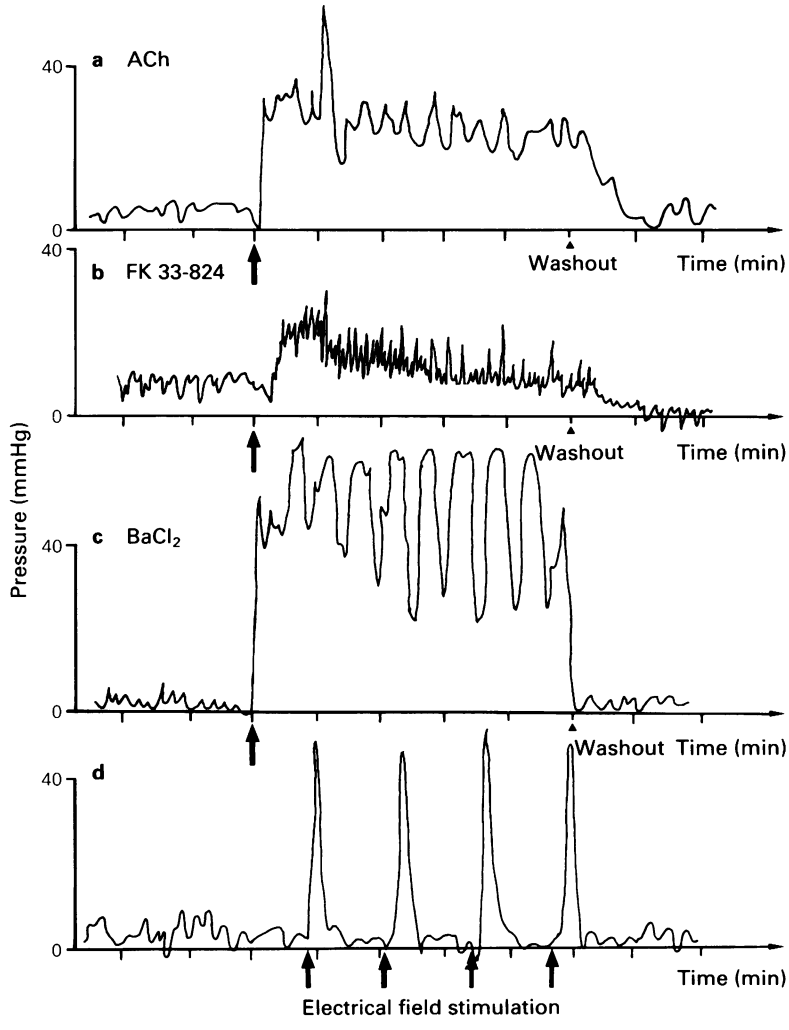

Figure 2 Intraluminal pressure recordings from proximal colonic segments of rats before and during different chemical stimuli and electrical stimulation. The arrows indicate the addition of each stimulus; (a) acetylcholine (10^{-4}M), (b) FK 33-824 (10^{-6}M), (c) barium chloride (10^{-3}M) and (d) electrical field stimulation.

pattern were observed in middle and distal colonic segments. Figure 3 shows the dose-response relationship of the effects of verapamil and pinaverium bromide on the integrated tonic colonic pressure of the proximal and middle colonic segments stimulated by 10^{-3}M barium chloride, 10^{-4}M acetylcholine or 10^{-6}M FK 33-824. Verapamil 10^{-7}M to 3×10^{-6}M dose-dependently inhibited the stimulation effects of these three drugs in both colonic segments. The estimated IC_{50}s of verapamil on the barium chloride-, acetylcholine- and FK 33-824-stimulated proximal colon were 1.2×10^{-7}M, 9.9×10^{-8}M and 1.1×10^{-7}M, respectively. The extent of stimulatory activity of acetylcholine and FK 33-824 was similar in all colonic segments, whereas the mid and distal colonic segments were significantly more sensitive to stimulation by barium chloride ($P < 0.005$). Pinaverium bromide also inhibited the effects of the three chemical stimuli on tonic colonic muscle contraction in a dose-related manner. The IC_{50}s of pinaverium bromide were 3.4×10^{-6}M for barium chloride stimulation, 5.2×10^{-6}M for acetylcholine and 3.3×10^{-6}M for FK 33-824 stimulation. This indicated that the inhibition induced by pinaverium bromide was about 30 times weaker than that of verapamil. In this series of experiments too the different sensitivity between the

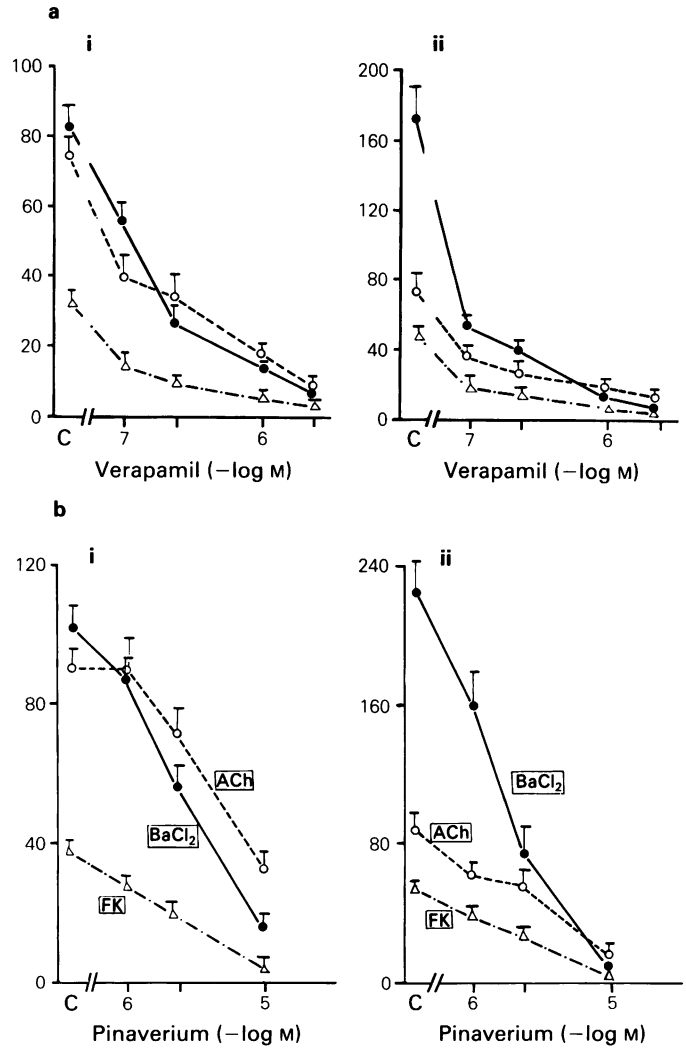

Figure 3 Inhibition by (a) verapamil or (b) pinaverium of integrated intraluminal colonic pressure responses (ICPR) of rat colon segments ((i) proximal colon and (ii) middle colon) to $BaCl_2$ (10^{-3}M; ●), acetylcholine (10^{-4}M; ○) or FK 33-824 (10^{-6}M; △). Vertical lines represent s.e.mean; $n = 12$. 'C' on horizontal axis refers to control responses in the absence of verapamil or prinaverium.

proximal and middle or distal colonic segment to barium chloride stimulation was observed.

Furthermore the slopes of the straight part of the barium chloride-inhibition curves obtained with pinaverium bromide and verapamil in the proximal but also in the middle colonic segment did not statistically differ from parallelism, which is compatible with a similar mode of action for pinaverium bromide and verapamil.

Both verapamil and pinaverium bromide dose-dependently inhibited the responses of the proximal colon to electrical field stimulation (Figure 4). The slopes of both curves are not statistically different from parallelism, thereby permitting the possibility of a similar inhibitory mechanism being involved. The estimated IC_{50} of pinaveriumn bromide was 6.8×10^{-6}M and that of verapamil 2.8×10^{-7}M.

In Figure 5 the inhibitory effects of verapamil and pinaverium bromide on the amplitudes of electrically-induced muscle contractions in the proximal colonic segment before and after increasing the calcium concentration in the Ringer solution are compared. A tenfold increase in the extracellular calcium concentration leads to similar reductions in the inhibitory effects

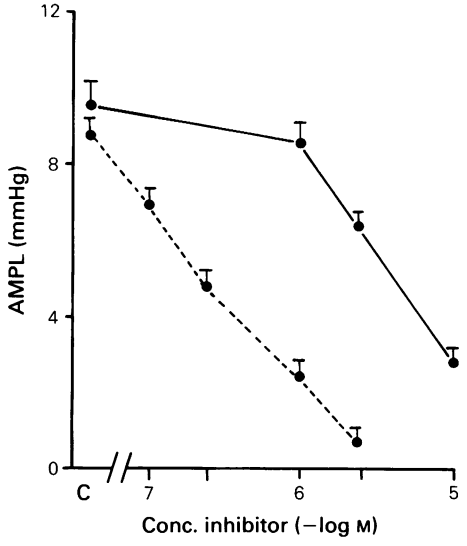

Figure 4 Inhibition by verapamil (● . . . ●) or pinaverium (● — ●) of peak amplitude of pressure (AMPL) responses of rat proximal colon to electrical field stimulation. Vertical lines represent s.e.mean; $n = 12$. 'C' on horizontal axis refers to control responses in the absence of inhibitor.

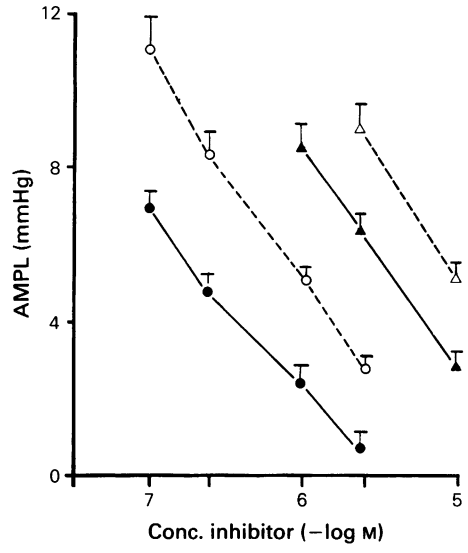

Figure 5 Inhibition by verapamil (●, ○) or pinaverium (▲, △) of peak amplitude of pressure (AMPL) responses of rat proximal colon to electrical field stimulation before (filled symbols) and after (open symbols) supplementation of the bath solution with exogenous calcium ions (18 mM $CaCl_2$). Vertical lines represent s.e.mean; $n = 12$.

of verapamil and pinaverium bromide without significantly affecting the slopes and the parallelism of the curves.

Discussion

The data obtained in this study demonstrate that pinaverium bromide dose-dependently inhibits contractile responses of rat colonic segments to the neurotransmitter acetylcholine, the [Met]enkephalin analogue FK 33-824, the heavy metal salt barium chloride and electrical field stimulation. The calcium antagonist verapamil exhibits similar inhibitory effects but is 30 times more potent than pinaverium bromide. This observation together with the fact that the inhibition curves of the responses to barium showed a parallel shift in response to verapamil and pinaverium bromide suggests that they induce their inhibitory effects by a similar mechanism probably via an effect on the calcium channels, which has been shown to be the case in other systems for verapamil (Droogmans *et al.*, 1983). This hypothesis is further corroborated by observations which indicate that barium ions are transferred through the calcium channels (Reuter *et al.*, 1982). Verapamil and pinaverium bromide also exhibited similar inhibitory effects on the responses to electrical field stimulation which supports the findings of Droogmans *et al.* (1983), who recognized an effect of these substances on voltage-dependent calcium channels. Furthermore, a ten fold increase in extracellular calcium counteracted to a similar extent the inhibitory effects of verapamil and pinaverium bromide on field-stimulated colonic segments. This finding is probably due to a greater influx of calcium through the calcium channels, as a result of the increased calcium gradient across the muscle cell membrane, and suggests the importance of calcium channels in the inhibitory action of pinaverium bromide.

Since there is evidence for an acetylcholine-independent action of FK 33-824 on rat colonic tone (Scheurer *et al.*, 1981), it is suggested that the similarity between the pinaverium bromide- and the verapamil-inhibition curves of the responses to acetylcholine and FK 33-824 is due to the effects of pinaverium bromide or verapamil on calcium channels, even though acetylcholine and FK 33-824 might activate calcium channels via different pathways.

In contrast to acetylcholine, FK 33-824 and field stimulation, barium chloride consistently induced tonic pressure responses in the middle or distal colonic segments which were about twice those elicited in proximal colonic segments. This might be explained by middle and distal segments possessing a greater number of calcium channels which could be passed by barium ions than the proximal colon.

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