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Choline is an inhibitory modulator of cholinergic nerve function in guinea-pig colon

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Abstract—Using the novel smooth muscle-myenteric plexus (smmp) preparation from the guinea-pig colon, it has been possible to measure acetylcholine (ACh) overflow by a radiolabelling technique. The possibility that choline may be a modulator of cholinergic nerve activity in this preparation was investigated by observing its effects on electrically-evoked overflow of [^{3}H]acetylcholine. Choline (72, 144 μM), in concentrations that did not contract the smmp preparation, caused a depression of electrically-evoked [^{3}H]ACh overflow. This effect was unlikely to be due to actions on cholinesterase enzymes by end product inhibition. Choline also produced substantial non-muscarinic elevation of spontaneous [^{3}H]overflow. It is concluded that inhibitory modulation of enteric cholinergic nerve activity by presynaptic muscarinic receptors may not be exclusively mediated by the actions of acetylcholine.

A new smooth muscle-myenteric plexus (smmp) preparation of the guinea-pig colon has been devised and shown to be well suited to studying cholinergic nerve activity when using a radiolabelling technique to estimate acetylcholine (ACh) release (Trout 1986). For electrical stimuli of more than one pulse it was thought possible that choline, derived from metabolized ACh, may be involved in modulating transmitter release from the nerve terminal. Such an effect of choline has been demonstrated in the guinea-pig longitudinal muscle-myenteric plexus preparation where choline reduces ACh output by stimulating presynaptic muscarinic receptors (Kilbinger & Kruel 1981). Thus the present study was undertaken to determine whether choline functions as an inhibitory modulator of cholinergic nerve activity in the guinea-pig colon.

Materials and methods

Guinea-pig colonic smmp strips were prepared and set up as previously described (Trout 1986). Strips were incubated for 60 min at 37°C in Krebs fluid containing 4 μCi mL^{-1} of [^{3}H]choline chloride (spec. act. 15 Ci mmol^{-1}, Amersham) after which they were superfused at 2·2–2·4 mL min^{-1} with Krebs fluid containing hemicholinium-3 (34·8 μM). Electrical field stimulation (EFS; 10 Hz, 0·5 ms at 150 mA) was carried out during the first 30 min of the incubation period to maximize pre-loading of releasable [^{3}H]ACh stores (Szerb 1976). Incubation and superfusion (by displacement overflow) of individual smmp strips were carried out in the same 1·5 mL volume baths which were fitted with vertical platinum wire electrodes to allow EFS of the tissue from a constant current stimulator. After 90 min equilibration the preparations were stimulated (1 Hz, 0·5 ms, 240 pulses at 150 mA). Superfusion fluid was collected for 2 min periods and 0·5 mL aliquots removed from each sample for liquid scintillation counting. Sample collections, to measure 'basal release', were made before and after both EFS and choline addition, and again at the end of the experiment. Collections were made during and immediately after EFS stimulation and during exposure of the tissue to choline to measure 'stimulated' release. Efficiency of counting was determined automatically by the external standard channels ratio method. Radioactive content of superfusion samples was expressed as Bq g^{-1} and calculated as described previously (Burleigh 1988). The evoked release of radioactive material, collected during and after EFS and during exposure to choline, was calculated from the difference between the 'calculated' basal release and the release during stimulation (calculated basal release + evoked release). Calculated basal release was obtained by fitting a regression line through observed basal values.

The E_2/E_1 ratio was calculated by dividing the sum of evoked values of the second period of EFS (E_2) by the sum of evoked values of the first period (E_1).

The evoked release of tritiated material by EFS, after previous incubation of the tissue with [^{3}H]choline, accurately represents the [^{3}H]ACh release from guinea-pig smmp strips (Trout 1989). Following a modification of the methodology of Marchbanks &

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Israel (1971) detailed in Burleigh & Trout (1986), it has been shown that evoked release of ^{3}H-material from the guinea-pig smmp preparation is due largely to the release of [^{3}H]ACh. Compared to pre-stimulus values, which were similar for both compounds, [^{3}H]choline content of samples collected during stimulation increased 18·6% while the [^{3}H]ACh content increased 270·2%.

Unless otherwise stated, data are expressed as medians with interquartile ranges. Statistical comparisons were made using the Mann-Whitney U test (2-tailed).

Results

The evoked overflow of radioactivity (10 min collection beginning with the stimulus) for the first stimulation period of six control experiments was 3·02 (4·00–2·44) kBq g^{-1} above the calculated basal line; calculated basal release for the same 10 min period was 2·97 (5·40–2·44) kBq g^{-1}. The tissue content at the beginning of the stimulation period was 495·0 (606·4–354·5) kBq g^{-1}.

Smmp strips were stimulated (1 Hz, 0·5 ms, 240 pulses at 150 mA) after the 90 min washout period, and again 44 min later. Choline (7·2 to 144 μM) was infused 30 min before the second period of stimulation and these experiments compared with those where no choline was added. Choline had a profound effect on spontaneous ^{3}H-overflow; in the first 8 min of infusion, choline displaced 165·0 (131·7–385·0; n = 6) Bq g^{-1}, 973·3 (626·7–1155·0; n = 6) Bq g^{-1} and 881·7 (813·3–1206·7; n = 5) Bq g^{-1} with 7·2, 72·0 and 144 μM, respectively, compared with saline control giving −63·3 (−116·7–80·0; n = 7) about the calculated basal line. This effect was also seen in the presence of atropine (0·3 μM, Table 1). As ^{3}H-overflow was still markedly elevated at the time of the second stimulation period (Fig. 1) the amount of radioactivity above the calculated baseline at the time of stimulation, and not fractional release, has been used as the measure of evoked [^{3}H]ACh overflow.

At the highest dose tested choline (144 μM) depressed electrically-evoked [^{3}H]ACh overflow (Fig. 2).

The effect of atropine on the actions of choline was assessed by introducing the antagonist 26 min before the first stimulation period and maintaining its presence for the duration of the experiments. Any effect of atropine on the evoked [^{3}H]ACh overflow will be accounted for using this design. The experimental design also incorporated a longer interval between stimulation periods (74 min) and a longer exposure time to choline (60 min) to more closely examine the time-course of its effect on spontaneous ^{3}H-overflow (Fig. 1). In this series of experiments choline (72 μM) depressed electrically-evoked [^{3}H]ACh overflow. This difference compared to the first series of experiments probably does not represent a time-dependent effect since, in that series, statistical significance was not reached on account of a single value approximately twice that of the rest of the group (n = 6). The effect of choline (72 μM) on evoked [^{3}H]ACh overflow in the presence of atropine (0·3 μM) was not significantly different from control experiments in the presence of atropine alone. In contrast to this atropine did not affect the action of choline on spontaneous ^{3}H-overflow (Table 1). No

Table 1. Interaction of choline (72 μM) and atropine (0·3 μM) on (A) spontaneous ^{3}H-overflow and (B) electrically-evoked [^{3}H]ACh overflow from the guinea-pig colonic smmp preparation. Atropine was infused 26 min before the first of the two stimulation periods, choline was introduced 60 min before the second stimulation period. For effects on spontaneous overflow data expressed as amounts of radioactivity detected (Bq g^{-1}) over the 60 min infusion; for effects on evoked overflow data expressed as the ratio of evoked overflow of radioactivity by the two stimulation periods (E_2/E_1).

	Drug regimen		
Control	Choline	Choline + Atropine	Atropine
(A) Spontaneous ^{3}H-overflow (above calculated baseline)			
152·3 (−649·5–227·3)	6200·0* (6251·7–6108·3)	5633·3* (6031·7–4830·0)	−500·0 (−337·3–585·0)
(B) Evoked [^{3}H]ACh overflow (E_2/E_1)			
0·73 (0·71–0·97)	0·65* (0·60–0·65)	0·66 (0·56–0·70)	0·67 (0·67–0·80)

n = 5 for each value, * = $P < 0·05$.

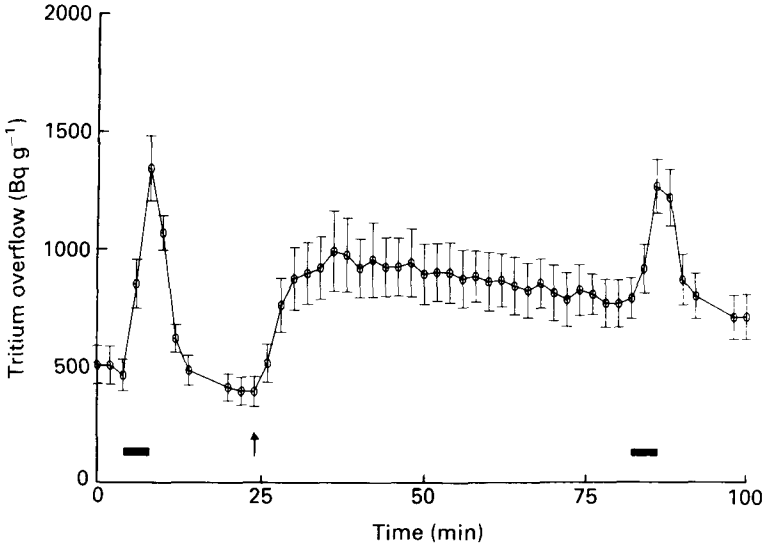

FIG. 1. Effect of choline (72 μM) and electrical field stimulation (1 Hz, 0·5 ms, 240 pulses at 150 mA) on ^{3}H- and [^{3}H]ACh overflow from tissues pre-loaded with [^{3}H]choline. Stimulation periods are indicated by horizontal bars, choline addition by the arrow after which the drug was left in contact with the tissue for the remainder of the experiment. Data expressed as means ± s.e.m., n = 5 for each point.

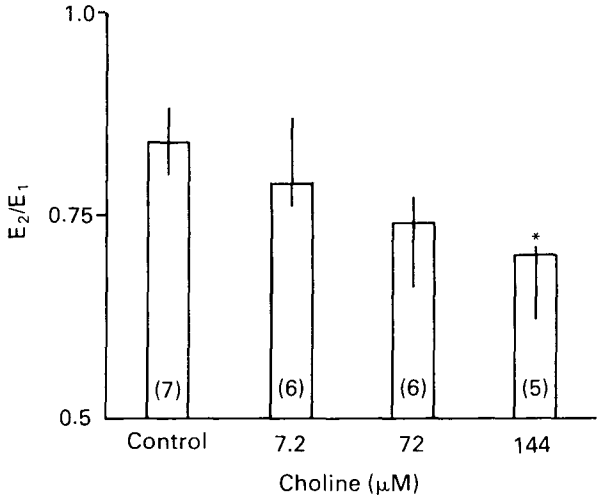

FIG. 2. The effect of choline on electrically-evoked [^{3}H]ACh overflow from the guinea-pig colonic smmp preparation. Choline was infused 30 min before the second of two stimulation periods 44 min apart. Data expressed as the ratio of evoked overflow of radioactivity of the two stimulation periods (E_2/E_1) and given as medians with interquartile ranges. Numbers of experiments in parentheses, * = $P < 0.05$.

contractions of the smmp preparation were observed at the doses of choline used.

Finally, to establish whether the actions of choline on evoked [^{3}H]ACh release were due to an anticholinesterase effect (by end-product inhibition), the effect of the compound on contractions of guinea-pig longitudinal muscle-myenteric plexus (lmmp) strips to ACh and bethanechol were evaluated. In the presence of choline (144 μM) ED50 values for ACh and bethanechol were reduced from 0.15 ± 0.03 to 0.08 ± 0.01 (n = 3) and from 5.02 ± 2.81 to 2.18 ± 1.01 (n = 5), respectively.

Discussion

Previous investigations have shown that the increased output of ^{3}H-material evoked by EFS from tissue pre-loaded with [^{3}H]choline was due largely to [^{3}H]ACh release whereas spontaneous ^{3}H-overflow consists of both [^{3}H]choline and [^{3}H]ACh (Wikberg 1977; Vizi et al 1984; Wetzel & Heller-Brown 1985; Burleigh & Trout 1986). This has been confirmed for the smmp preparation. Further, since the increase in radioactivity measurements coincided with either choline or ACh spots upon TLC analysis, this indicates that the tritium label remains attached to the choline moiety.

A concentration-dependent effect of choline on spontaneous ^{3}H-overflow has also been reported by Kilbinger & Kruel (1981). These authors considered the effect reflected displacement, by choline, of [^{3}H]ACh or [^{3}H]choline from the tissue. The present experiments showing insensitivity of the effect to atropine is consistent with the argument that the elevation of spontaneous ^{3}H-overflow is not connected with a muscarinic receptor-mediated mechanism.

The depression of electrically-evoked [^{3}H]ACh release, by choline, and its prevention by atropine is indicative of an action of choline at presynaptic muscarinic receptors. Similar conclusions were arrived at by Kilbinger & Kruel (1981). That choline can act as a 'cold carrier' to augment the efficiency of tritium recovery, may result in the measurement of evoked [^{3}H]ACh being an overestimate. Thus the depression of evoked [^{3}H]ACh by choline may be larger than it appears from this type of experiment.

An alternative explanation of the effects of choline on evoked [^{3}H]ACh overflow was the possibility that choline may inhibit cholinesterase enzymes via 'end-product inhibition'. This would increase the synaptic acetylcholine concentration and facilitate activation of inhibitory autoreceptors. That inhibitors of cholinesterase enzyme activity reduce the output of acetylcholine to a given stimulus is well documented (Kilbinger & Wessler 1980; Dunant & Walker 1982; Trout 1989). To investigate this possibility the actions of choline were assessed on contractile responses of lmmp strips to two muscarinic agonists: acetylcholine, which is destroyed by cholinesterase enzymes, and bethanechol which is not. The ED50 values of both agonists were reduced by a comparable extent and while an increase in the potency of acetylcholine is consistent with an anti-cholinesterase action of choline, the effect seems to be the result of another action since the potency of bethanechol was similarly affected.

The present experiments and those of Kilbinger & Kruel (1981) reveal a possible alternative mechanism for autoreceptor modulation of acetylcholine release from enteric cholinergic nerves. In the broader sense there may be implications regarding the use of choline to enhance cholinergic transmission in the central nervous system (Hollander et al 1986).

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