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An investigation of the mechanism involved in the cholinergic action of meptazinol

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In concentrations above 20 μM, (±)-meptazinol produced a contraction of the guinea-pig isolated ileum and this effect was antagonized by atropine (0·01 to 0·3 μM) in a manner which was not competitive. Cooling the preparation to 15 °C blocked the contractile action of meptazinol and of dimethylphenylpiperazinium (DMPP) but did not affect the action of carbachol. Twitch responses of the rat phrenic nerve-diaphragm preparation induced by indirect electrical stimulation in the presence of naloxone (20 nM) were potentiated by meptazinol (1 to 40 μM) which also reversed a partial blockade of the twitch induced by tubocurarine. Neither of these effects was seen in tissues which had been pretreated with the cholinesterase inhibitor BW284C51 (0·2 μM) though tetraethylammonium iodide (40 μM) was still able to enhance the responses to stimulation. In the presence of naloxone (20 nM) electrically induced responses of the rat isolated rectum were abolished by cinchocaine (10 μM), partially blocked by atropine (0·1 to 0·4 μM) and potentiated by meptazinol (1 to 30 μM). The latter action was not seen when meptazinol was administered in the presence of BW284C51. It is concluded that the cholinergic action of meptazinol in these tissues is due to an indirect effect, probably involving inhibition of cholinesterase and that no evidence was seen of any ability to increase the release of acetylcholine itself.

The opioid partial agonist meptazinol (Stephens et al 1978; Green 1983) is known to bind to μ-opioid receptors (Blurton et al 1982) but it is thought that a cholinergic component may also contribute to its analgesic action since this latter effect is partially antagonized by anticholinergic drugs such as atropine and scopolamine (Bensreti et al 1983; Bill et al 1983). The cholinergic action is particularly evident in isolated ileal preparations where meptazinol produces potentiation of electrically induced twitch responses in contrast to the inhibition produced by other opioid drugs (Duchesne et al 1984). The mechanisms involved in this cholinergic effect are unclear as meptazinol was thought to lack anticholinesterase properties (Stephens et al 1978), but more recent work has shown that cholinesterase is inhibited (Galli 1985; Strahan et al 1985), the (−)-enantiomer being about 100 times less potent than physostigmine (Ennis et al 1986). In addition it has been reported that meptazinol is able to increase the K^+-evoked overflow of tritium from mouse cortical slices previously incubated with [^{3}H]choline (Ennis & Stephens 1984) but it is uncertain if this is a consequence of cholinesterase inhibition or a reflection of an ability of meptazinol to increase the neuronal release of acetylcholine itself.

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This paper reports an investigation of the mechanism of the cholinergic action of meptazinol in isolated tissues.

METHODS

Guinea-pig isolated ileum (with or without field stimulation)

Recordings were made isotonically as described in detail by Duchesne et al (1984). Mepyramine (0·1 μM), hexamethonium (60 μM; except in experiments with DMPP) and naloxone (20 nM) were present at all times in the physiological saline. Temperature was normally maintained at 36 °C but some experiments were carried out at 15 °C. When appropriate, electrical stimulation was applied using rectilinear pulses (0·1 Hz, 1 ms, supramaximal voltage). Agonists were added to the tissue bath at 5 min intervals (meptazinol 10 min) and allowed to remain in contact with the tissue until a maximum effect had been produced. In experiments at 15 °C these times were doubled. When required, atropine was added to the saline and allowed to equilibrate with the tissue for at least 40 min.

Rat phrenic nerve-diaphragm preparation

The preparation was set up as described by the Staff of the Dept of Pharmacology, University of Edinburgh (1970). Naloxone (20 nM) was present in the saline for the duration of the experiment. A resting

tension of 1 g was used, recordings were made on a Devices MX2 recorder and stimulation was applied at 0·1 Hz (20 μs, supramaximal voltage). Meptazinol was added to the preparation at 45 min intervals and allowed to remain in contact with the tissue for 15 min or until a maximum response was produced, whichever was the shorter. When appropriate BW284C51 was allowed to equilibrate with the tissue for 50 min before meptazinol was added.

Rat isolated rectum preparation (Shaw 1979)

Male Wistar rats (235–410 g) were killed by a blow on the head. A 2 cm portion of the rectum was removed, cleared of adherent tissue and mounted in a tissue bath in a physiological saline (NaCl 118; KCl 3·7; $MgSO_4$ 1·17; $CaCl_2$ 2·5; KH_2PO_4 1·18; $NaHCO_3$ 25; glucose 11 mM also containing naloxone 20 nM; gassed with 95% O_2/5% CO_2; 35 °C) with a platinum electrode passing through the lumen of the rectum and the second electrode placed parallel to the first, about 2 cm distant. Electrical stimulation employing rectilinear pulses at 2·5 Hz (0·8 ms duration, supramaximal voltage) was applied to the tissue for 5 s every 40 s. Changes in length of the preparation were recorded isotonically (load 1 g). Reproducible responses were established after about 1 h. Meptazinol was added to the tissue bath using the cumulative method and responses were measured after a contact time of 7·5 min. BW284C51 was added to the physiological saline and allowed to equilibrate with the tissue for 40 min.

Drugs used

Atropine sulphate (Sigma), carbachol chloride (BDH), cinchocaine hydrochloride (Ciba), 1,5-di(*p*-*N*-allyl-*N*-methylaminophenyl)pentan-3-one dibromide (BW284C51; Wellcome), dimethylphenylpiperazinium iodide (DMPP; Sigma), hexamethonium bromide (Sigma), (±)-meptazinol hydrochloride (Wyeth), mepyramine maleate (Sigma), naloxone hydrochloride (Sigma), neostigmine bromide (Koch-Light), physostigmine sulphate (Sigma), tetraethylammonium chloride (TEA; BDH), tubocurarine chloride (Sigma).

Statistical procedures

Where appropriate all results are expressed as mean ± standard error (n = number of observations contributing) and statistical significance was obtained using Student's *t*-test.

RESULTS

Guinea-pig isolated ileum

In concentrations below 10 μM, meptazinol increased the electrically induced twitch responses without affecting resting tension. At concentrations of 20 μM and above, twitch tension was still increased initially but in addition a concentration-related contractile response was seen.

In unstimulated preparations, a similar contractile response was observed (20 to 200 μM) and the size of the maximal response produced by meptazinol was similar to that produced by carbachol (0·1 to 0·5 μM). Atropine (0·01, 0·03, 0·1, 0·3 μM) antagonized the response to carbachol shifting the curve in a parallel manner without reducing the maximal response. Analysis of these data using the method of Arunlakshana & Schild (1959) yielded log (DR − 1) against log (antagonist concentration) plots with an average slope of 1·08 ± 0·09 (n = 6) which is not significantly different from unity ($P > 0·4$). The pA_2 value obtained was 8·86 ± 0·12 (n = 6). In each of 4 experiments atropine antagonized the contractile response to meptazinol but these data were not amenable to treatment by the method of Arunlakshana & Schild since the curves were non-parallel and a considerable reduction in maximal response was produced (Fig. 1). In five experiments hexamethonium (80 μM) did not affect the response to carbachol or to meptazinol but reduced the response to DMPP yielding a dose-ratio of 10·5 ± 1·8 (n = 5).

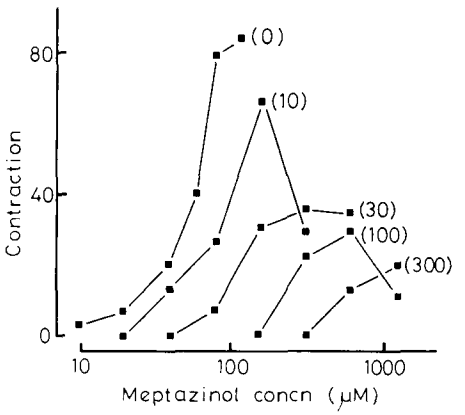

FIG. 1. Typical examples of concentration-response curves from guinea-pig isolated ileum for meptazinol in the presence of atropine at various concentrations (figures in parentheses; nM).

In each of 6 experiments cooling the preparation to 15 °C produced little change in the size of the response to carbachol but greatly reduced the

responses to DMPP (1 to 20 μM) and to meptazinol (Fig. 2). The effect was reversed when the temperature was restored to 36 °C.

Physostigmine, neostigmine and BW284C51 all produced a contracture of the ileum upon which was superimposed considerable and variable spontaneous activity. A similar type of response was seen in tissues exposed to high doses of meptazinol.

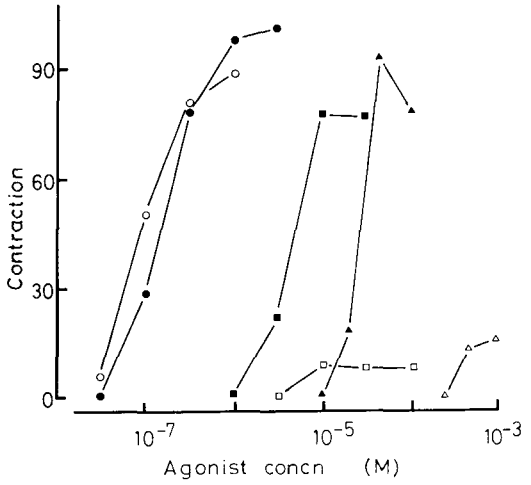

FIG. 2. Typical example of concentration-response curves from guinea-pig isolated ileum for carbachol (●), dimethylphenylpiperazinium (DMPP; ■) and meptazinol (▲). Data obtained at 36 °C and at 15 °C are represented by filled and open symbols, respectively.

The rat phrenic nerve-diaphragm preparation

Meptazinol (1 to 40 μM) potentiated the electrically induced twitch responses; the effect could be reversed by washing and was reproducible when doses were given at 45 min intervals over 6 h. The greatest increase produced by a concentration of meptazinol was expressed as a percentage of the initial twitch tension immediately before the addition of meptazinol. The maximal effect varied considerably between preparations but averaged 38 ± 11% (n = 6). At concentrations above 100 μM the potentiation produced by meptazinol was followed by a slow decline in the size of the twitch which continued for at least 30 min and was unaffected by the addition of a high concentration of naloxone (0·2 μM) (Fig. 3). The initial twitch tension and the potentiation of the twitch were very variable between preparations and in order to combine data from different preparations the maximum potentiation produced by meptazinol in an otherwise untreated tissue was taken as the maximum response.

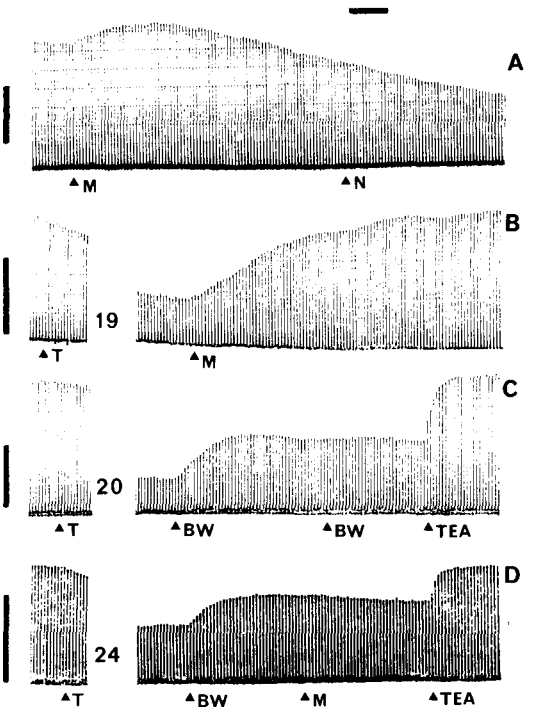

FIG. 3. Showing twitch responses of the rat isolated diaphragm induced by electrical stimulation (0·1 Hz, 20 μs, supramaximal voltage) of the phrenic nerve. For each record the vertical bar represents an increase in tension of 0·5 g and the horizontal bar a time period of 2 min. All concentrations are expressed in μM and are given below. Trace A shows the effect of (±)-meptazinol (M), at a high concentration (400 μM). At N the concentration of naloxone was raised from 0·02 to 0·2 μM. Traces B, C and D show the effect of meptazinol (M; 20 μM), BW284C51 (BW; 0·2 μM) or tetraethylammonium iodide (TEA; 400 μM) on a partial blockade induced by tubocurarine (T; 0·2 μM). The numbers between the first and second portions of these records show the elapsed interval in minutes.

Potentiations produced in the same preparation by lower concentrations of meptazinol both in the presence and absence of BW284C51 were expressed as a percentage of this value and are shown in Fig. 4. Clearly, meptazinol produced a concentration-dependent potentiation which was very much less in the presence of BW284C51. TEA (400 μM) always produced an increase in the twitch response even when added in the presence of a maximally effective concentration of meptazinol.

Tubocurarine (0·2 μM) produced a partial blockade of the twitch response which was reversed by meptazinol (20 μM) alone (Fig. 3) but not when the partial blockade was produced after the prior administration of BW284C51 (0·2 μM). This concentration of BW284C51 was chosen since it was effective in potentiating the twitch response during a partial

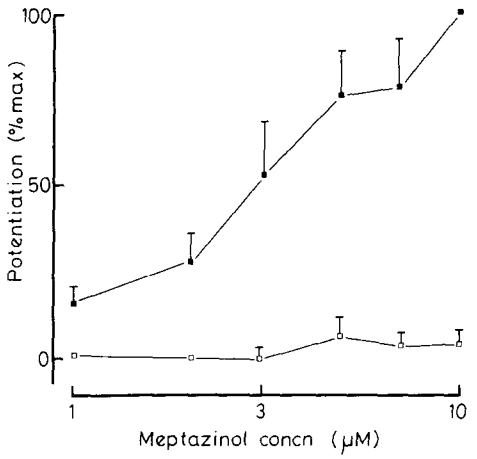

FIG. 4. Showing the potentiation (expressed as a percentage of the maximum increase) produced by meptazinol of the electrically induced twitch responses of the rat phrenic nerve diaphragm preparation. The points represent means (n = 6) and the bars standard errors (omitted when falling within the area of the point). Open points show the effect in the presence of BW284C51 (0·2 μM) and are statistically significantly different ($P < 0.05$; Student's t-test), except at 1 μM, from the effect produced at corresponding concentrations in the absence of BW284C51.

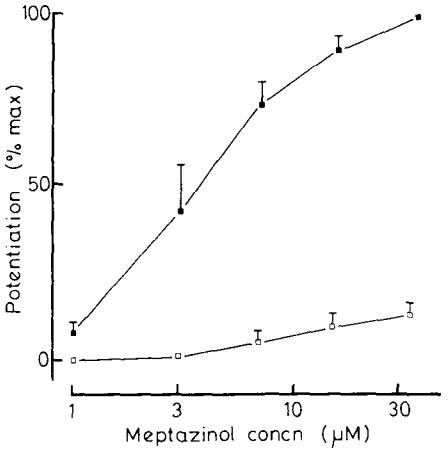

FIG. 5. Showing the potentiation (expressed as a percentage of the maximum increase) produced by meptazinol of the electrically-induced twitch responses of the rat isolated rectum preparation. The points represent means (n = 4) and the bars standard errors (omitted when falling within the area of the point). Open points show the effect in the presence of BW284C51 (0·2 μM) and are statistically significantly different ($P < 0.05$; Student's t-test), except at 1 μM, from the effect produced at corresponding concentrations in the absence of BW284C51.

tubocurarine blockade, and doubling the concentration produced no further potentiation (Fig. 3). TEA (400 μM) potentiated the twitch response when given alone, in the presence of tubocurarine and meptazinol or BW284C51, or in the presence of a combination of the two latter agents (Fig. 3).

The rat isolated rectum

Electrically induced twitch responses of the rat isolated rectum were abolished by cinchocaine (10 μM) and were reduced by between 30 and 60% by atropine in a concentration-dependent manner though concentrations of atropine higher than 0·4 μM produced no greater reduction in response.

Meptazinol (1 to 30 μM) potentiated the twitch response and though the effect was variable between preparations an average maximal potentiation of 65 ± 11% (n = 6) was produced. In the presence of BW284C51 (0·2 μM) the effect of meptazinol was significantly reduced (Fig. 5) though TEA (400 μM) still potentiated the twitch response.

Meptazinol (10 μM), BW284C51 (0·2 μM) and TEA (400 μM) all reversed the inhibition of the twitch response produced by atropine and TEA was still effective in the presence of either meptazinol or BW284C51. However, in the presence of BW284C51 meptazinol was no longer effective.

DISCUSSION

The ability of meptazinol to contract the ileum is mediated through muscarinic receptors since the effect is antagonized by atropine at low concentrations. It seems unlikely that this is a direct agonist effect since the antagonism by atropine did not show competitive characteristics, i.e. the maximal response was reduced. The observation that low temperatures also antagonized the effect (and that of the known indirectly acting agonist DMPP) while leaving the response to the directly acting agonist carbachol unaffected, also suggests that meptazinol does not affect the muscarinic receptors directly. Attempts to investigate the action of meptazinol on ileum in the presence of anticholinesterase agents were abandoned when it was found that anything but the shortest exposure to these agents produced a progressively developing contracture and considerable spontaneous activity. This made the tissues impossible to work with and it was for this reason that experiments were continued on diaphragm and rectum which did not show this effect. Meptazinol potentiated the twitch responses of the diaphragm in response to stimulation of its cholinergic motor nerves and reversed a partial blockade induced by tubocurarine. Both these effects are consistent with an increased functional availability of acetylcholine.

The fact that meptazinol no longer produced these actions when cholinesterase was inhibited by BW284C51 suggests that this increase in the availability of acetylcholine is a result of cholinesterase inhibition. It could be argued that a maximal twitch response had been produced but the addition of TEA (which increases the release of acetylcholine; Collier & Exley 1963) potentiated the response still further making this explanation unlikely.

In the rectum the situation is more complex since a cholinergic as well as a non-cholinergic transmitter is clearly involved. Nevertheless, responses induced by stimulation of the autonomic nerves in the rectum were potentiated by meptazinol and meptazinol reversed the effects of atropine. Neither of these effects were seen in the presence of the anticholinesterase agent BW284C51, while the actions of TEA were still apparent.

No evidence has been found of any action of meptazinol on cholinergic nerves which cannot be accounted for by its known ability to inhibit cholinesterase. Indeed, the concentration range which induced cholinergic affects in all three preparations (1 to 10 μM) corresponds well with the EC50 (6·4 ± 2·5 μM) reported by Ennis et al (1986) for inhibition of cholinesterase activity. It is possible that some additional action is produced on neurons in the CNS which is not seen on motor or autonomic cholinergic nerves in peripheral tissues, but we have no evidence from this work that meptazinol produces cholinergic effects by any mechanism other than cholinesterase inhibition.

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