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On the Mechanism Involved in the Ability of Meptazinol to Potentiate the Effects of Sympathetic Nerve Stimulation

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Abstract—Mouse isolated vas deferens responded to field stimulation (0·1 Hz) with twitch responses which were abolished by $\alpha\beta$-methyleneadenosine 5′-triphosphate (0·5 μM) and were potentiated 2 to 3 fold by meptazinol (5–300 μM). Exogenous adenosine 5′-triphosphate (4–30 μM) also caused a twitch response but was unaffected by meptazinol (30 μM) as was the response to phenylephrine. The effect of meptazinol on the electrically-induced twitch was reproducible, fast in onset, easily reversed by washing and was still seen in the presence of prazosin (1 μM), yohimbine (1 μM), propranolol (0·1 μM), atropine (0·1 μM), physostigmine (1 μM), cocaine (1 μM) or desmethylimipramine (0·3 μM) indicating that the mechanism involved does not depend on adrenoceptors, cholinergic mechanisms or blockade of uptake$_1$. Mouse isolated atria responded to stimulation (1, 2 or 5 Hz) of their sympathetic nerves via transmural electrodes with chronotropic responses which were abolished by atenolol (5 and 50 μM) but were unaffected by $\alpha\beta$-methyleneadenosine 5′-triphosphate (0·5 μM). Meptazinol (100 μM) failed to potentiate the responses. It is suggested that meptazinol potentiates the effects of the non-adrenergic non-cholinergic transmitter thought to be involved in the response of the mouse vas deferens to electrical stimulation.

The opioid partial agonist meptazinol (Stephens et al 1978) binds to μ-opioid receptors (Blurton et al 1984) but a cholinergic component may contribute to its analgesic action since this latter effect is partially antagonized by anticholinergic drugs (Bill et al 1983). The cholinergic action is particularly evident in isolated ileal preparations where meptazinol produces potentiation of electrically induced twitch responses rather than the inhibition usually seen with opioid agonists (Duchesne et al 1984) and is probably due to inhibition of cholinesterase (Ennis et al 1986; Hetherington et al 1987).

Meptazinol also potentiates electrically induced responses of some sympathetically innervated tissues such as mouse vas deferens (Duchesne et al 1984) and this paper reports an investigation of the mechanism involved.

Materials and Methods

Animals

Male mice (T.O. strain; 30–55 g) were stunned and killed by cervical dislocation.

Mouse isolated vas deferens

The vas deferens was removed, cleared of adherent tissue and mounted in physiological saline (NaCl 128, KCl 5·63, $CaCl_2$ 2·16, NaH_2PO_4 1·19, $NaHCO_3$ 25, glucose 11·1, sucrose 13·1 mM also containing naloxone 20 nM, gassed with 5% carbon dioxide in oxygen).

Changes in length of the tissue in response to electrical stimulation (40 V, 2 ms duration, 0·1 Hz) were recorded isotonically (load 150 mg). The tissue bath was drained and refilled every 5 min except when concentration-response curves to meptazinol were being determined. Reproducible control twitch responses were established after about 45 min and all responses in the presence or absence of drugs were expressed as a percentage of these control twitches. Meptazinol was added in a cumulative manner and responses were allowed to reach equilibrium (usually <2 min) before the next dose was added. $\alpha\beta$-Methyleneadenosine 5′-triphosphate ($\alpha\beta$-MeATP) was added to the tissue bath and allowed to act for 3 min; other drugs were added to the bulk supply of physiological saline and allowed to remain in contact with the tissue for at least 20 min before effects were recorded or other doses of meptazinol added.

ATP or phenylephrine were added to the tissue bath every 3 or 5 min, respectively, allowed to remain in contact with the tissue for 10 or 30 s before washing and contractile responses were recorded isometrically (initial resting load 100 mg). When appropriate meptazinol was added to the tissue bath 2·5 min before each dose of ATP or phenylephrine.

Mouse vas deferens previously incubated with [^{3}H]noradrenaline

The method is detailed elsewhere (Goodall et al 1984). Briefly, a vas deferens was incubated with [^{3}H]noradrenaline for 45 min and then washed every 2 min for 60 min. Electrical stimulation (40 V, 400 mA, 2 ms duration, 2·5 Hz for 90 s every 14 min) was applied through parallel platinum wire electrodes. The effluent from the tissue bath was counted for tritium and the fractional resting and fractional evoked overflows were determined. All results are expressed as the ratio (Sx/S2) of the value obtained in later stimulation periods (Sx) to that obtained in the control stimulation period (S2) performed at the start of the experiment after an initial stimulation period (S1; results discarded).

Mouse isolated atria

Paired atria were suspended in physiological saline as above (containing atropine 0·5 μM) at 35°C. Spontaneous contrac-

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tions were recorded isometrically (resting load 50 mg) and displayed on a Devices recorder. Atrial rate was derived from the tension record. After 30 min equilibration transmural electrical stimulation was applied for 10 s (1 ms duration; 400 mA; 1, 2, 5 or 20 Hz) through parallel platinum wire electrodes every 15 min and the tissue was then washed by drainage. In some tissues meptazinol, $\alpha\beta$-MeATP or atenolol was added to the tissue bath 2, 3 or 10 min, respectively, before a period of stimulation.

Drugs used

Adenosine 5′-triphosphate (disodium salt; Sigma), atenolol (Sigma), atropine sulphate (Sigma), cocaine hydrochloride (Boots), desmethylimipramine hydrochloride (Geigy), meptazinol hydrochloride (Wyeth), $\alpha\beta$-methyleneadenosine 5′-triphosphate (lithium salt; $\alpha\beta$-MeATP) (Sigma), naloxone hydrochloride (Sigma), phenylephrine hydrochloride (Sigma), prazosin (Pfizer), propranolol hydrochloride (ICI), physostigmine sulphate (Sigma) and yohimbine hydrochloride (Sigma). [^{3}H](-)-noradrenaline (30 Ci mmol^{-1}) was obtained from Amersham International Ltd.

Statistical procedures

Where appropriate, all results are expressed as mean ± standard error (n = number of observations contributing) and tests for statistical significance utilized paired or unpaired *t*-tests as appropriate.

Results

Effects on twitch response of vas deferens to electrical stimulation

At concentrations between 5 and 300 μM, meptazinol produced a concentration-dependent potentiation of the response to electrical stimulation of up to 3-fold at the highest concentration. The effect was fast in onset, usually reaching equilibrium within 2 min, and was completely reversed on washing the tissue for 15 min. Reproducible responses were obtained when a tissue was exposed repeatedly to a concentration of meptazinol of 40 μM.

Physostigmine (1 μM), atropine (0·1 μM), propranolol (0·1 μM), prazosin (1 μM) and yohimbine (1 μM) did not affect the size of the twitch response when administered alone and in their presence the effect of meptazinol was not changed in a statistically significant manner (Fig. 1). Desmethylimipramine (DMI) (3, 30 and 300 μM) reduced the size of the twitch response to 66 ± 4%, 38 ± 6% and 26 ± 5% of the control responses, respectively (n = 4); in the presence of 300 μM DMI, meptazinol still potentiated the twitch response through the magnitude of the effect was somewhat smaller than that produced by meptazinol alone. Cocaine (1 μM) also reduced the size of the twitch response but in the presence of cocaine the potentiation produced by meptazinol was only marginally reduced (Fig. 2).

In each of 4 experiments 0·5 μM $\alpha\beta$-MeATP produced a short-lived contractile response and then abolished the twitch response of the vas deferens to electrical stimulation within 3 min. This effect was slowly reversible by repeated washing for 20 to 60 min.

ATP (4 to 30 μM) produced a transient contractile response which reached peak tension in 1–2 s. The contraction diminished to about 50% of its peak value within a further 2 s. Responses were reasonably reproducible but were highly dependent on the speed of dose administration, the position of the syringe needle in the tissue bath and the vigour of the oxygenation (mixing). Clearly an equilibrium situation was not even approached. The responses to ATP were little

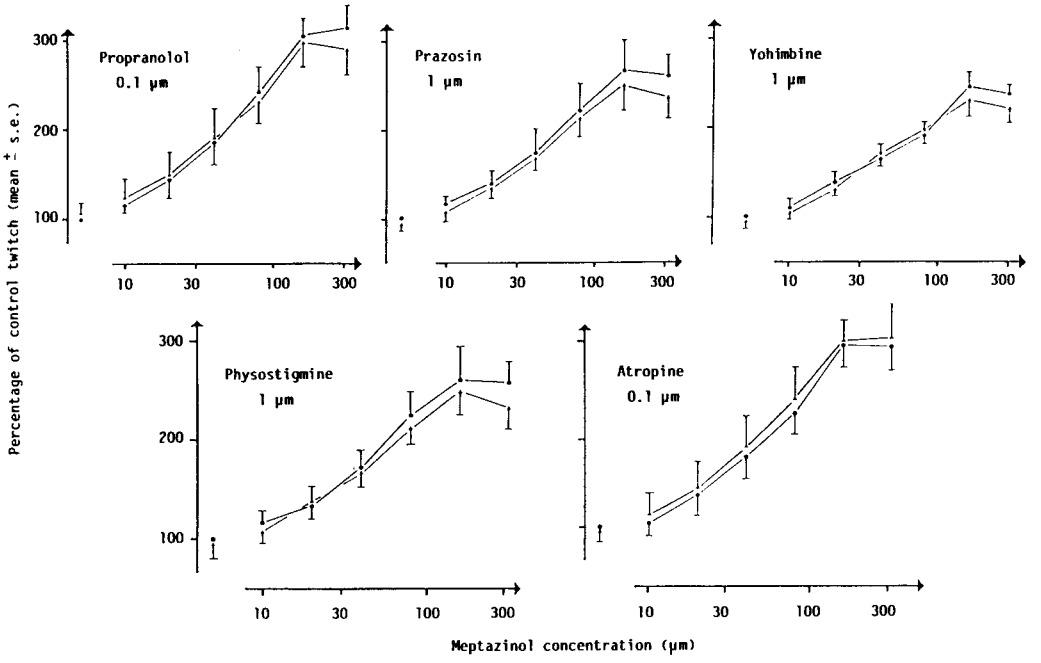

FIG. 1. Effect of meptazinol (μM), alone (●———●) and in the presence of various drugs (▼———▼) on the size of the twitch response of mouse isolated vas deferens to electrical stimulation (0·1 Hz, 2 ms, 40 V). Four tissues contributed to each mean value and the bars represent standard errors. All results are expressed as a percentage of the initial control twitches obtained before any drugs were added (●) and the effect on twitch size of the appropriate drug alone is shown (▼). The effect of meptazinol was not changed in a statistically significant manner at any point ($P > 0.05$; paired *t*-test).

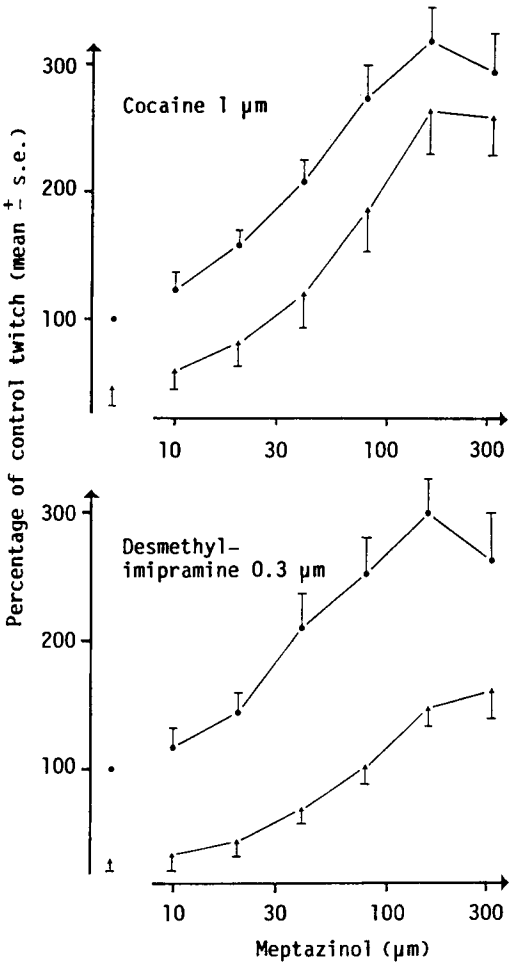

FIG. 2. Effect of meptazinol (μM), alone (●———●) or in the presence (▲———▲) of cocaine (upper; n = 7) or desmethylimipramine (lower; n = 5) on the size of the twitch response of mouse isolated vas deferens to electrical stimulation (0·1 Hz, 2 ms, 40 V). Each value is a mean and the bars represent standard errors. All results are expressed as a percentage of the initial control twitches obtained before any drugs were added (●) and the effect of the appropriate drug alone (▲) is shown. The effect of the drug is Table 1. Values (mean ± s.e.) of various parameters in two groups (n = 5) of mouse isolated vas deferens incubated with [^{3}H]noradrenaline and then subjected to electrical stimulation.

changed in the presence of meptazinol. The ratio of the equi-effective dose of ATP in the absence to that in the presence of meptazinol (30 μM) averaged 0·95 ($\log_{10}$dose ratio $-0·023 \pm 0·185$; mean ± s.e.; n = 7; this value is not statistically significantly different from zero; $P > 0·05$; t-test). In 2 experiments contractile responses to phenylephrine were little affected by meptazinol (30 μM) the equi-effective dose-ratios being 1·4 and 0·78 though the general variability in the size of the responses elicited even by repeated administration of the same dose of phenylephrine detracts from the value of these data.

Effect on resting and evoked tritium overflow from vas deferens

Two groups of tissues were used; one a control group and the other exposed to various concentrations of meptazinol. There was no statistically significant difference between the two groups in terms of the tissue content of tritium, the resting or the evoked tritium overflow (Table 1). In control

Table 1. Values (mean ± s.e.) of various parameters in two groups (n = 5) of mouse isolated vas deferens incubated with [^{3}H]noradrenaline and then subjected to electrical stimulation.

Parameter	Control group	Group to be treated later with meptazinol
Tissue content (d min^{-1})	440 007 ± 20 209	406 831 ± 49 510
Resting overflow (d min^{-1})	3410 ± 196	3143 ± 393
Evoked overflow (d min^{-1})	4498 ± 725	3634 ± 591
Fractional resting overfow (× 10^3)	7·87 ± 0·23	7·84 ± 0·06
Fractional evoked overflow (× 10^3)	9·95 ± 1·42	9·12 ± 0·77

There is no statistically significant difference between corresponding values ($P > 0·05$).

tissues the Sx/S2 ratio for both resting and evoked fractional overflow of tritium fell slightly over the 6 periods of stimulation. Meptazinol produced little effect on resting or evoked overflow except at the highest concentration (300 μM) where the Sx/S2 ratios show that resting overflow was increased while evoked overflow was reduced. These changes were small but were statistically significant ($P < 0·05$) (Fig. 3).

Effect on isolated atria

The rate and force of spontaneous atrial beat was not significantly different ($P > 0·4$) in two groups of tissues

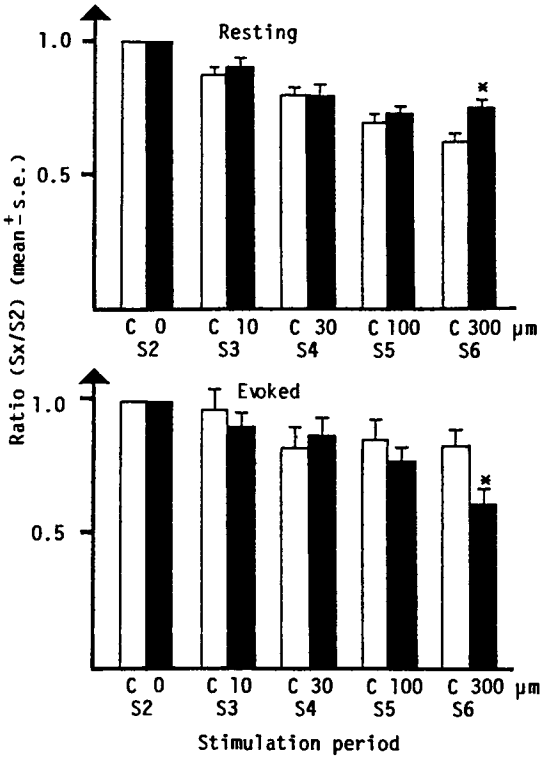

FIG. 3. Effect of meptazinol on the resting (upper) and electrically evoked (lower) fractional overflow of tritium from mouse isolated vas deferens previously incubated with [^{3}H]noradrenaline. The columns show the ratio of the overflow in an initial period (S2) to that in successive later periods (S3 to S6) when meptazinol was present (solid columns; meptazinol concentration shown in μM) or absent (open columns; C). The asterisk show values statistically significantly different from the untreated tissues ($P < 0·05$).

(387±14 and 384±12 beats min^{-1}; 31±5 and 40±9 mg, respectively). Both groups (n=8 in each case) responded to electrical stimulation with a positive chronotropic response. In control tissues a second set of stimulations evoked chronotropic responses which were generally smaller than those evoked by the first set but the difference was not statistically significant ($P>0.2$). Addition of meptazinol (100 μM) evoked consistent negative chronotropic and positive inotropic responses which averaged 17±3% and 37±5%, respectively when expressed as a percentage of the values immediately before addition of meptazinol. In tissues in which a second set of stimulations was performed in the presence of meptazinol (100 μM) there was a smaller chronotropic response at each frequency tested (Fig. 4).

In comparison with control tissues the chronotropic response evoked by stimulation at 5 or 1 Hz was not significantly affected by $\alpha\beta$-MeATP (0·5 μM) but was practically abolished by atenolol (5 or 50 μM) (Table 2).

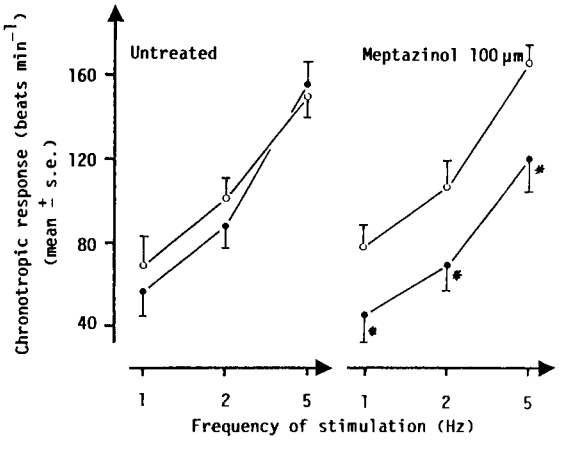

FIG. 4. Chronotropic responses (beats min^{-1}) evoked by electrical stimulation (1 ms duration, 400 mA, 10 s) at 5, 2 or 1 Hz of mouse isolated paired atria. Tissues (n=8 in each group) were subjected to an initial series of stimulations (○———○) in the absence of meptazinol and then to a second series (●———●) with meptazinol (100 μM) present or absent (untreated). In untreated tissues stimulation at 20 Hz evoked a chronotropic response which averaged 220±12 beats min^{-1} (mean±s.e.; n=8). The asterisks indicate mean values statistically significantly different when compared with their own control ($P<0.05$; paired t-test).

Table 2. Effect of atenolol and $\alpha\beta$-methyleneadenosine 5′-triphosphate ($\alpha\beta$-MeATP) on chronotropic responses of mouse isolated atria to electrical stimulation (10 s of 1 ms duration 400 mA pulses). At each frequency (5 and 1 Hz), the chronotrophic response evoked by a second period of stimulation (in the presence of drugs as shown) has been expressed as a percentage of that evoked before exposure to drugs. Values are means±s.e. and the number of observations contributing to each value is shown in parentheses.

	Drug treatment			
Frequency Hz	None —	Atenolol 5 μM	Atenolol 50 μM	$\alpha\beta$-MeATP 0·5 μM
5	95±2 (8)	6±12 (7)	7±7 (8)	95±5 (8)
1	82±10 (8)	9±11 (7)	10±13 (8)	87±17 (5)

Discussion

Since all experiments were conducted in the presence of naloxone it is unlikely that any of the effects observed are due to an action of meptazinol on opioid receptors. The pA_2 of naloxone against μ-opioid receptors is about 8·7 (Duchesne et al 1984) and the opioid agonist action of meptazinol on mouse vas deferens is in any case weak (Goodall et al 1986). Neither is the potentiation of the response to electrical stimulation of the mouse vas likely to be mediated through any action on cholinesterase or through muscarinic receptors since neither physostigmine nor atropine themselves produced potentiation and neither did they modify the effects of meptazinol. The concentration of physostigmine used would be expected to be highly effective against cholinesterase since an IC50 of 67 nM has been reported (Ennis et al 1986) and atropine is generally accepted as having a pA_2 of about 9 against muscarinic receptors. The mechanism by which sympathetic responses are potentiated is therefore different to that by which parasympathetic responses are potentiated.

Neither β- nor α-adrenoceptors can be involved since neither propranolol nor prazosin themselves affected the twitch response or the action of meptazinol. It might initially seem surprising that yohimbine, which is known to block presynaptic α_2-adrenoceptors and to increase noradrenaline release at the concentration used (Goodall et al 1984), did not potentiate the effects of electrical stimulation as reported by Blakeley et al (1988). However whereas Blakeley used a stimulation frequency of 5 Hz, the low rate of stimulation used here (0·1 Hz) effectively produces twitch responses to single shock stimulation and modulation of transmitter release through presynaptic α_2-adrenoceptors will be minimal under these conditions. The lack of effect of yohimbine applied alone and its failure to modify the effects of meptazinol indicate that the action of meptazinol is not mediated through α_2-adrenoceptors.

Both cocaine and DMI produced inhibition of the twitch response to electrical stimulation when applied alone in contrast to the potentiation produced by meptazinol. This observation itself suggests that inhibition of uptake$_1$ by meptazinol cannot be the cause of the potentiation. Since both uptake blockers reduced the twitch size this complicates an assessment of their effect on the response to meptazinol but it is clear that meptazinol can still produce a considerable potentiation even when uptake$_1$ is substantially blocked.

The determination of the overflow of tritium from tissues previous incubated with [^{3}H]noradrenaline is not necessarily an accurate measure of noradrenaline release since metabolites as well as [^{3}H]noradrenaline will contribute (Marshall 1983). Nevertheless, the fact that evoked tritium release was not increased by concentrations of meptazinol which produced considerable potentiation of the twitch response argues against the effect of meptazinol being due to an increase in noradrenaline release. The difference in experimental conditions should be noted however in that twitch responses were effectively to single shock stimulation (0·1 Hz) while measurements on tritium release were made over a period of 90 s stimulation at 2·5 Hz.

In contrast to its effects on vas deferens, on isolated atria meptazinol did not produce potentiation of the submaximal chronotropic responses evoked by 10 s trains of 1, 2 or 5 Hz

sympathetic stimulation. These chronotropic responses were unaffected by $\alpha\beta$-MeATP and were mediated though β-adrenoceptors since they were abolished by atenolol. This observation is compatible with the direct evidence that noradrenaline release in vas deferens is also not increased. It seems more likely that the potentiation of the twitch responses of the vas deferens is due to an action on the non-cholinergic non-adrenergic transmitter, probably ATP, thought to be co-released with noradrenaline in this tissue. Abolition of the twitch response of the vas deferens by $\alpha\beta$-MeATP is in agreement with the findings of Blakeley et al (1988). ATP is not thought to serve a functional role in the response of atria to sympathetic stimulation and the lack of effect of $\alpha\beta$-MeATP supports this. It must be remembered that the results with exogenous ATP were obtained under conditions which did not approach equlibrium and this detracts from their reliability. Similarly, though the lack of effect on responses to phenylephrine suggests excitability of the muscle fibres is not increased, the variability in the responses makes it impossible to draw this conclusion firmly. However, the lack of effect of meptazinol on exogenous ATP suggests the potentiating action of meptazinol against electrically induced twitches is due to increased release of ATP though we have no direct evidence that exogenous and endogenous ATP would necessarily behave similarly. If this is so it is surprising that noradrenaline release is not also increased since co-release of ATP and noradrenaline are thought to occur from the same vesicle. There is evidence however, that noradrenaline and ATP release from myenteric plexus varicosities can be affected independently by clonidine (Hammond et al 1988) and we are currently investigating this possibility in mouse vas deferens.

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