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Effect of Meptazinol on Evoked Responses in Rat Vas Deferens

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Abstract—Responses of rat isolated vas deferens to electrical stimulation through field electrodes (400 mA, 1 ms duration, single shocks at 5 min intervals) were potentiated by meptazinol (10 to 300 μM) in whole tissues and also in the separated prostatic and epididymal portions. The effect was fast in onset, reproducible and easily reversed by washing. Prazosin (0·1 μM) practically abolished the response of the epididymal portion to electrical stimulation while the response of the prostatic portion was only slightly reduced (< 20%). In the presence of prazosin, meptazinol still produced potentiation of the response of the prostatic portion. Nifedipine (2 μM) practically abolished the response of the prostatic portion to electrical stimulation while the response of the epididymal portion was only slightly reduced (< 20%). In the presence of nifedipine, meptazinol no longer produced potentiation of the response of the epididymal portion. Exogenous ATP (5 μM to 1 mM) and phenylephrine (1 to 50 μM) produced a contractile response which was potentiated in the presence of meptazinol (100 μM) but in the presence of meptazinol (100 μM) and nifedipine (5 mM) together, potentiation of phenylephrine no longer occurred. It is suggested that potentiation by meptazinol of electrically induced responses in this tissue is due to a direct action on the smooth muscle.

Pharmacological investigations of opioid agonists often utilize isolated tissues such as ileum and vas deferens where electrically evoked contractile responses are inhibited by activation of μ-, δ- and κ-opioid receptors. However, the opioid partial agonist meptazinol (Stephens et al 1978) is unusual in that it potentiates electrically evoked twitch responses in guinea-pig isolated ileum and mouse vas deferens (Duchesne et al 1984). The effect on isolated ileum is probably due to inhibition of cholinesterase (Ennis et al 1986; Hetherington et al 1987) but the mechanism by which meptazinol potentiates the effects of stimulation of the sympathetic innervation of mouse vas deferens is not clearly established. It is possible that the potentiation is due to an effect on the non-adrenergic non-cholinergic (NANC) transmitter, thought to be ATP (see Blakeley et al 1988); little of the potentiation of neuronally induced responses appears to be mediated through an action involving noradrenaline (El-Mas et al 1989).

In rat vas deferens, the contribution of the NANC transmitter and of noradrenaline to the electrically evoked response can be isolated from each other, at least partially, by dividing the vas deferens into two halves. In the prostatic end the NANC transmitter causes the major part of the response while in the epididymal end noradrenaline is the major transmitter (McGrath 1978; Brown et al 1983). This paper now reports an investigation of the effects of meptazinol on these two components of the response of the rat vas deferens to electrical stimulation.

Materials and Methods

Male albino Wistar rats (200–355 g) were killed by a blow on the head. The vas deferens was removed and the serous coat stripped off. In some experiments, the tissue was cut into two portions of equal length each comprising about 45% of the vas deferens; the middle 10% was discarded. The tissue was mounted in physiological saline at 38°C (NaCl 119, KCl 4·7, $CaCl_2$ 2·5, $NaHCO_3$ 25, KH_2PO_4 1·2, glucose 11·1 mM and naloxone 20 nM; gassed with 5% carbon dioxide in oxygen). A resting tension of 0·5 g was applied and changes in tension were recorded isometrically (Lectromed UF 1) and displayed on a Lectromed MX 216 recorder.

Electrical stimulation (rectilinear pulse 1 ms duration, 400 mA) was applied every 5 min via field electrodes located above and below the tissue. The tissue bath was drained and refilled at least every 10 min and drugs were generally added to the tissue bath immediately after each wash. Where a fixed concentration of nifedipine or prazosin was used for a large proportion of the experiment, the drug was added to the bulk supply of physiological saline. In all experiments utilizing nifedipine illumination was provided by a sodium light. Reproducible responses were established after about 40 min and in each experiment the magnitude of these responses was taken as a control and all subsequent responses were expressed as a percentage of this control value. When necessary meptazinol was added to the tissue bath immediately after a stimulus and allowed to remain in contact with the tissue for 10 min before its effects were determined.

ATP was injected as rapidly as possible into the tissue bath (which was aerated vigorously to promote rapid mixing), allowed to remain in contact for 10 s and then the tissue was washed several times. Phenylephrine was allowed to remain in contact for 30 s before washing. Usually a dose interval of 5 min was used. Meptazinol, with or without nifedipine was added to the tissue bath as required 4 min before the next dose of agonist.

Statistical treatment

Where appropriate, values are given as mean ± standard error and the number of observations contributing to each

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mean is shown (n). Tests for statistical significance utilised paired or unpaired Student's *t*-tests as appropriate.

Drugs used

Adenosine-5′-triphosphate (lithium salt; Sigma), meptazinol hydrochloride (Wyeth), naloxone hydrochloride (Sigma), nifedipine (Bayer), phenylephrine hydrochloride (Sigma), prazosin (Sigma). Stock solutions were made in distilled water except for prazosin (5% glucose + 5% glycerol in water) and nifedipine (dimethylsulphoxide); dilutions were prepared in physiological saline.

Results

Whole vas deferens

Electrical stimulation of the isolated whole vas deferens evoked a consistent contractile response which was biphasic; one peak occurring about 250 ms after the stimulus and a second peak or clear shoulder at about 650 ms. Meptazinol (10 to 300 μM) increased the size of both peaks by up to 3-fold at the highest concentration (Fig. 1). The effect was fast in onset, easily reversed by washing and was reproducible.

Bisected preparations

The prostatic and epididymal portions showed monophasic responses to electrical stimulation which peaked at about 250 and 650 ms after stimulation, respectively. The responses were potentiated by meptazinol over a similar concentration range as was effective in the whole vas deferens (Fig. 2) and this effect was reproducible. In both prostatic and epididymal portions (n = 6 in each group) there was no statistically significant difference ($P > 0.2$; paired *t*-test) at any point between an initial concentration-effect curve for the action of meptazinol and a second similar curve determined 1 h later.

Electrically evoked twitch responses were unaffected by appropriate amounts of the vehicle solutions used to dissolve nifedipine and prazosin. Prazosin (0·01 to 1 μM) reduced (and at the higher concentrations practically abolished) the response of the epididymal portion to electrical stimulation.

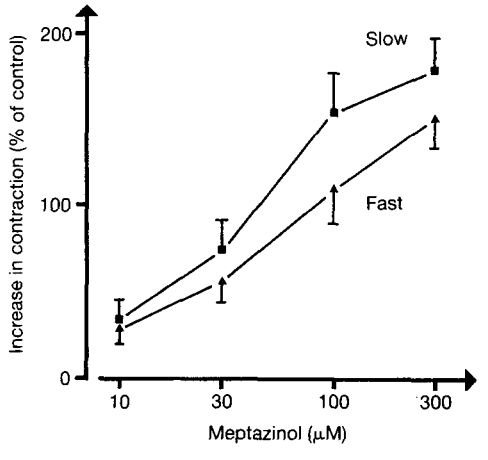

FIG. 1. Effect of meptazinol on the fast (250 ms) and slow (650 ms) contractile responses evoked by single shock (400 mA, 1 ms duration pulses every 5 min) field stimulation of rat isolated whole vas deferens. Responses are expressed as a percentage of control responses obtained before the addition of drugs. Values are means and the bars show the standard error (n = 5).

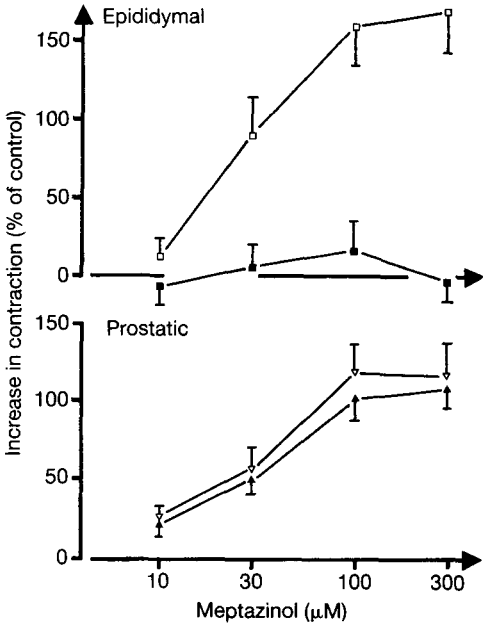

FIG. 2. Effect of meptazinol on response of portions of rat isolated vas deferens to field stimulation (400 mA, 1 ms duration every 5 min). Upper; epididymal portion in the absence (□) and presence (■) of nifedipine (2 μM). Lower; prostatic portion in the absence (▽) and presence (▲) of prazosin (0·1 μM). Responses are expressed as a percentage of control responses before the addition of drugs. The values are means and the bars show the standard error (n = 6).

The response of the prostatic portion was only slightly reduced (< 20%) (Fig. 3) at the lower concentrations and higher concentrations produced no additional effect. In the presence of prazosin (0·1 μM) the effect of meptazinol on the prostatic portion was not altered in a statistically significant manner (Fig. 2). Nifedipine (0·1 to 10 μM) reduced (and at the higher concentrations practically abolished) the response of the prostatic portion to electrical stimulation. The response of the epididymal portion was only slightly reduced (< 20%) (Fig. 3) at the lower concentrations and higher concentrations produced little further effect. In the epididymal portion in the presence of nifedipine (2 μM) the response to meptazinol was abolished and at none of the concentrations tested was a statistically significant potentiation produced ($P > 0.05$) (Fig. 2).

Response to exogenous agonists

Exogenous ATP (5 μM to 1 mM) evoked a short-lived contractile response which reached a maximum in less than 2 s and was not maintained. The response was dose-dependent and reproducible dose-response curves could be obtained. These curves where shifted to the left when re-determined in the presence of meptazinol (100 μM) and the size of the maximum response which could be elicited was increased, though the magnitude of this effect was variable from tissue to tissue, as can be seen from the standard errors in Fig. 4. Phenylephrine (1 to 50 μM) induced a somewhat stronger maximal contraction than did ATP but was also potentiated by meptazinol to a similar extent. A paired *t*-test showed that meptazinol produced an increase in the size of the response to both agonists which was statistically significant at each dose level used ($P < 0.001$ in every case). In the

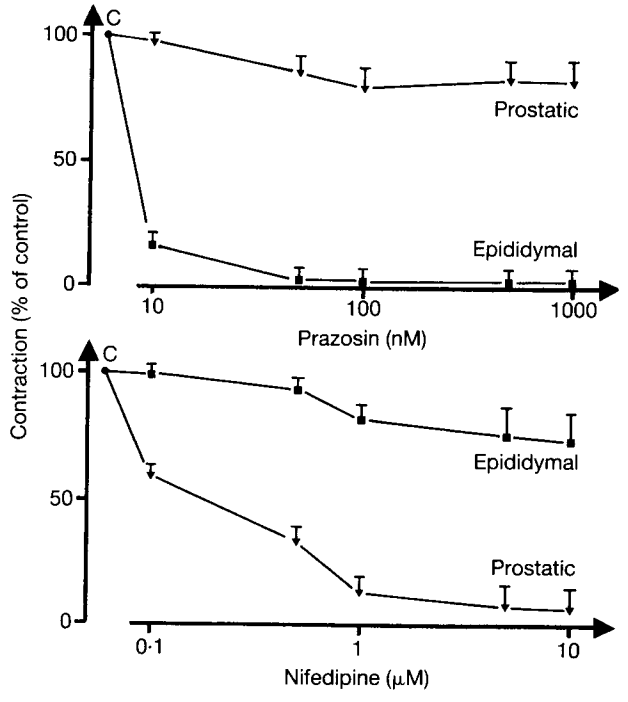

FIG. 3. Effect of prazosin (upper) and of nifedipine (lower) on responses of prostatic (▼) or epididymal (■) portions of rat isolated vas deferens to field stimulation (400 mA, 1 ms duration every 5 min). Responses are expressed as a percentage of control responses (C) obtained before the addition of drugs. The values are means and the bars show the standard error (n = 6).

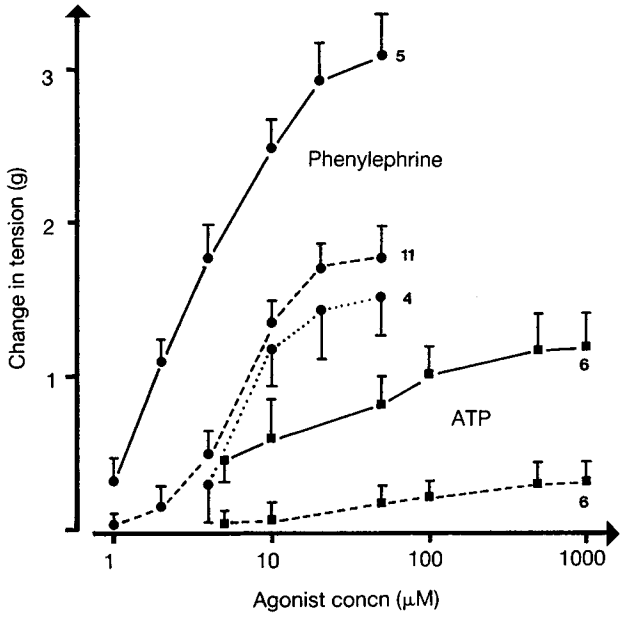

FIG. 4. Contractile responses of rat isolated vas deferens elicited by phenylephrine (circles) or ATP (n = 6) alone (– – –) or in the presence of meptazinol (100 μM; ——) or in the presence of meptazinol (100 μM) and nifedipine (5 μM) together (· · · · ·). The values are means and the bars show the standard error. The number of tissues contributing to each graph is shown in parentheses.

presence of meptazinol (100 μM) and nifedipine (5 μM) together, the response to phenylephrine was no longer potentiated ($P < 0.05$ at each concentration).

Discussion

Throughout this work stimuli were applied at 5 min intervals to avoid the complications resulting from the operation of endogenous feedback mechanisms which may be activated by trains of pulses. McGrath (1978) has shown that interaction between pulses does not take place when intervals of 2 min or more are used. As these workers have shown, the response of the rat whole vas deferens to electrical stimulation has two components; both appear to be potentiated by meptazinol over a similar concentration range to that which is effective in mouse vas deferens. The two components of the response of the rat vas deferens can be partially isolated by bisecting the tissue into a prostatic portion (where the NANC component predominates) and an epididymal portion (where transmission is mainly noradrenergic).

This separation is not complete however and further separation of the two components can be achieved by the use, in the epididymal portion, of nifedipine which blocks the effects of the NANC transmitter leaving a noradrenergically mediated response. Similarly, the use of prazosin in the prostatic portion blocks any residual noradrenergically mediated effects leaving the NANC mediated response little affected (Brown et al 1983). The experiments with various concentrations of prazosin suggest that in the prostatic portion there is a small residual noradrenergically mediated component in the electrically evoked response. This is abolished by prazosin leaving a purely NANC transmitter mediated response. Similarly, nifedipine slightly reduced responses of the epididymal portion, suggesting a small NANC transmitter mediated component is present. This is eliminated in the presence of nifedipine, leaving a purely noradrenergically mediated response in the epididymal portion. When meptazinol was applied under these conditions, only responses mediated by the NANC transmitter were potentiated, those responses which were purely noradrenergically mediated were unaffected. This result is in agreement with previously reported work on mouse vas deferens, where direct measurement of evoked tritium overflow in tissues incubated with [^{3}H]noradrenaline showed that meptazinol did not increase tritium overflow even in concentrations which were highly effective in potentiating the electrically-induced twitch responses (El-Mas et al 1989). However, meptazinol clearly potentiated responses to both exogenous ATP and to phenylephrine which suggests the effect is post-synaptic, non-specific and directly on the smooth muscle. If this is so it would be expected that responses evoked by endogenous noradrenaline would also be potentiated but this was not seen when meptazinol was applied in the presence of the calcium channel blocker, nifedipine. This could be explained if meptazinol had an effect on the handling of calcium; this would also account for the increase in the maximal response to exogenous agonists and the absence of noticeable potentiation of the response to phenylephrine when meptazinol (100 μM) and nifedipine (5 μM) were applied together. A direct effect on smooth muscle has been suggested to be involved in the action of meptazinol

on guinea-pig sphincter of Oddi (in-vitro) (Wade et al 1987) but in neither rat rectum nor diaphragm (where cholinergically mediated responses are potentiated by the anticholinesterase action of meptazinol) was any effect produced by meptazinol when cholinesterase was previously inhibited by BW284C51 (1,5-di(*p*-*N*-allyl-*N*-methylaminophenyl) pentan-3-one dibromide) (Hetherington et al 1987). Furthermore, in guinea-pig ileum the effect of direct electrical stimulation of the smooth muscle was not potentiated by meptazinol. Any effect on calcium handling or on smooth muscle sensitivity directly cannot therefore be entirely non-specific.

Acknowledgements

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