M₁ Receptor Agonist Activity Is Not a Requirement for Muscarinic Antinociception

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ABSTRACT

The analgesic effects of a series of muscarinic agonists were investigated by use of the mouse acetate acid writhing, grid-shock, hot-plate and tail-flick tests. The compounds tested were oxotremorine, pilocarpine, arecoline, acetylcholine, RS86 and four 3-3(substituted-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydroy-1-methyl pyridines (substituted TZTP), these being propoxy-TZTP, 3-Cl-propylthio-TZTP, xanomeline (hexyloxyl-TZTP) and hexylthio-TZTP. These agonists were also assayed for their ability to displace [³H]oxotremorine-M and [³H]pirenzepine binding and for their functional selectivity at pharmacologic M₁, M₃ and M₅ receptors. These compounds all produced dose-dependent antinociceptive effects in all of the mouse analgesia tests. The effects of oxotremorine in the writhing test were fully antagonized by the muscarinic antagonist scopolamine (0.1 mg/kg), but only partially antagonized by methscopolamine (10 mg/kg) and unaffected by the opioid antagonist naltrindone. 3-Cl-propylthio-TZTP and propoxy-TZTP had virtually no effect at the M₁ receptor subtype as measured by the human m₁ clone expressed in baby hamster kidney cells or the rabbit vas deferens assay. These compounds, however, were more potent in the analgesia tests than the selective M₁ agonists xanomeline and hexylthio-TZTP. These data suggest that muscarinic analgesia is mediated by central muscarinic receptors. However, activity at the M₁ receptor subtype is not a requirement for antinociceptive activity.

It has been known for more than 50 years that acetylcholinesterase inhibitors such as physostigmine have analgesic properties in man (see review by Hartvig et al., 1989; Peterson et al., 1986). The analgesic properties of these compounds lead to the suggestion that cholinergic mechanisms are involved in pain and analgesia.

Several studies have demonstrated that muscarinic cholinergic agonists as well as cholinesterase inhibitors are active in animal tests for analgesic activity (Chen, 1958; Herz, 1962; Metz et al., 1969; Harris et al., 1969; Ireson, 1970). Harris et al. (1969) showed that the nonselective muscarinic agonist oxotremorine and the cholinesterase inhibitor physostigmine were as efficacious as morphine in the mouse tail flick, but were 250 and 35 times more potent. Furthermore, acetylcholine administered intracerebroventricularly produced analgesia in the mouse tail-flick test (Pedigo et al., 1975). These analgesic effects were antagonized by muscarinic antagonists but not by opioid antagonists (Pedigo et al., 1975). These findings demonstrated that muscarinic analgesia is mediated directly through muscarinic receptors and not indirectly through opioid systems. In addition, chronic oxotremorine and physostigmine infusions have been shown not to produce physical dependence (Widman et al., 1985).

Although the accumulated data suggest that muscarinic agonists may be a viable alternative to opioid analgesics, the prominent parasympathomimetic cholinergic side effects such as bradycardia, hypotension, diarrhea, urination, salivation and lacrimation preclude their clinical utility. The recent discovery of subtypes of muscarinic receptors (Bonner et al., 1987), however, has provoked interest in the discovery of analgesic muscarinic agonists which are devoid of the unwanted effects of compounds such as oxotremorine.

Several studies have tried to investigate the muscarinic receptor subtypes involved in the antinociception evoked by muscarinic agonists. Ghelardini et al. (1990) suggested that M₁ receptors were involved, whereas Dawson et al. (1991) suggested that antinociception in the mouse tail flick was mediated by M₁ or M₃ receptors. More recently, Iwamoto and Marion (1993), working with intrathecally injected muscarinic agonists, suggested that M₁ and/or M₂ receptor subtypes were involved.

In the present study we report on the antinociceptive ef-

ABBREVIATIONS: TZTP, (1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methyl pyridine; substituted TZTPs, 3-(3-substituted-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methyl pyridines; DMEM, Dulbecco’s Modified Eagle’s Medium; HEPES, N-(2-hydroxyethyl)piperazine-N’-2-ethanesulfonic acid; BHK, baby hamster kidney.
fects of a range of cholinergic muscarinic agonists and show that these effects are mediated by central muscarinic receptors. Furthermore, we report on the antinociceptive effects of a range of new cholinergic agonists, substituted TzTPs, with varying functional subtype selectivity. We have investigated the selectivity of these compounds in cloned human m1 receptors, the rabbit vas deferens (M1; Shannon et al., 1993) and the isolated guinea pig atria (M2; Clague et al., 1985). Although the guinea pig ileum contains 70% M2 and 30% M3 receptors (Ford et al., 1991), contraction of this tissue has been shown to be mediated by M3 receptors (Michel and Whiting, 1988; Ford et al., 1991; Eglen and Harris, 1993; Honda et al., 1993; Doods et al., 1994). Therefore, we have used the guinea pig ileum to investigate activity of the compounds at M3 receptors. The data obtained with these compounds suggest that the antinociceptive effects of muscarinic compounds may not reside solely in their effects at the M1 subtype. Preliminary portions of these data have been partially published in abstract form (Swedberg et al., 1993).

Methods

Receptor Binding Studies

The method used has been fully described by Sauerberg et al. (1991). Fresh cortex from male Wistar rats was homogenized for 5 to 10 sec in 10 ml of 20 mM HEPES (pH 7.4). The suspension was centrifuged for 15 min at 4000 × g. The pellet was washed three times with buffer and centrifuged. The final pellet was homogenized in 20 mM HEPES (pH 7.4; 100 mg of tissue) and used for binding. For [3H]Hoxotremorine-M binding, 25 μl of test solution and 25 μl of [3H]oxotremorine-M (1.0 nM final concentration) were added to 0.5 ml of homogenate, and the solution was mixed and incubated for 30 min at 25°C. Nonspecific binding was determined in triplicate with 1 μM atropine (1993) and 1 μM MgCl2, 0.6; and glucose, 7.7. Yohimbine (1.0 μM) was included to block alpha-2 adrenergic receptors. The pH of the Krebs’ solution was maintained at 7.4 during all experiments by constant bubbling with 95% O2/5% CO2. Each segment was suspended between two platinum/iridium electrodes in a 10-ml organ bath maintained at 31°C and attached to a Grass PT03 force transducer. A passive force of 0.75 g was applied followed by an equilibration time of 45 min before continuous stimulation with square wave pulses at 0.05 Hz, 40 to 50 V, 0.5-msec duration. An equal number of prostatic and epididymal segments were used for each treatment. Changes in isometric tension were recorded and analyzed with an M5000 Signal Processing Center with XYZ real-time software (Modular Instruments, Inc., Malvern, PA) and a Compaq Deskpro 386 computer (Compaq Computer Corporation, Houston, TX).

Guinea pig ileum. Male Hartley guinea pigs (Mollegård, Ry, Denmark) were sacrificed by cervical dislocation. The hearts were quickly removed and the atria dissected free from the surrounding tissue. The atria were suspended in 10-ml organ baths and bathed in modified Krebs-Henseleit solution of the following composition (mM): NaCl, 118; NaHCO3, 25; KCl, 4.7; CaCl2, 2.5; MgCl2, 2.1; NaH2PO4, 1.03; and glucose, 11. The solution was continuously bubbled with 95% O2 and 5% CO2 and maintained at 37°C. The mechanical activity of the tissue was measured by a Hogan Sachs Electronics (March-Hugstetten, Germany) type 351 isometric force transducer connected via a HSE type 301 bridge amplifier to a Kontron type 340 potentiometric pen recorder.

The negative inotropic effect of compounds was determined by a sequential dosing regimen with a dosing interval of 30 sec. Responses were expressed as percentage reduction of the force of contraction during a 5-min control period before the application of the test compound. IC50 values were determined by standard line regression techniques.

Guinea pig ileum. Ileas were isolated from male Hartley guinea pigs (Mollegård, Ry, Denmark) that were sacrificed by cervical dislocation, the terminal 15 cm of ileum removed, and 1.5- to 2.0-cm lengths prepared and mounted in 10-ml organ baths containing Tyrode solutions of the following composition (mM): NaCl, 137; KCl, 2.68; CaCl2, 0.9; MgCl2, 1.05; NaHCO3, 11.9; NaHPO4, 0.42; and glucose, 5.55. The solution was continuously bubbled with 95% O2 and 5% CO2. Resting tension was set at 1 g and the tissue left to equilibrate for 1 hr. The mechanical activity of the tissue was measured by a Hugo Sachs Electronics (March-Hugstetten, Germany) type 351 isometric force transducer connected via a HSE type 301 bridge amplifier to a Kontron type 340 potentiometric pen recorder.

The contractile effect of compounds was investigated by a sequential dosing regimen with a 30-sec contact time for the compound and a 3-min dosing interval. The force of contraction in grams was measured 30 sec after addition of compound. EC50 values were calculated using standard linear regression techniques.

Analgesic Studies

Acetic acid-induced writhing. Separate groups of 5 to 10 mice (Crl:CF1®BR, Charles River, Portage, MI) each were administered...
vehicle or a dose of the test compound (s.c.), followed 25 min later by an intraperitoneal injection of 0.5% acetic acid. Each mouse was then placed in an individual clear plastic observational chamber and the total number of writhes made by each mouse was counted between 5 and 10 min after acetic acid administration (30–35 min after vehicle or test compound). Data are expressed as the mean number of writhes during the 5 min observation period. ED₅₀ values were determined by standard linear regression techniques.

**Grid-shock.** This part of the study was carried out according to the method of Swedberg (1994). Groups of 10 mice (male NMRI, Møllegaard, Denmark) were placed individually in transparent acrylic chamber (13 × 13 × 13 cm) equipped with a stainless steel grid floor through which electric shocks could be delivered. The top of the chamber was covered with a removable acrylic plate and had a decibel meter (precision sound level meter, type 2232, Bruel and Kjær, Copenhagen, Denmark) attached. A shock generator delivering current intensity increasing from 0.01 to 0.5 mA over a period of 30 sec was connected to the grid floor. Square wave pulses of 2 msec duration were presented at 30 Hz. A predrug latency (sec) to vocalization (a 70-dB squeak turned off the shocks) was generated for each mouse. A trial was always terminated at the 70-dB squeak level or after 30 sec (cutoff time), whichever came first. After the predrug trial, the mice were injected with drug or vehicle subcutaneously and retested 30 min later to produce a postdrug latency to vocalization. The pre- and postdrug scores were summarized over the 10 mice in each group and means and standard errors calculated. Percent analgesia was calculated by the following formula: (postdrug latency – predrug latency/cutoff time – predrug latency) × 100.

**Hot-plate.** Groups of 10 mice (male NMRI, Møllegaard, Denmark, 10–22 g) were placed individually on a hot plate maintained at 55°C, and the latency to licking of the front paws was measured in each mouse; animals not responding were removed after 30 sec (cutoff time). After this predrug trial, animals were injected with vehicle or drug s.c. and retested 30 min later to give a postdrug latency. Each animal was used only once. The pre- and postdrug latencies were summarized for the 10 mice in each group and the percentage analgesia calculated with the following formula: (Postdrug latency – predrug latency/cutoff time – predrug latency) × 100.

**Tail-flick.** Groups of 10 mice (male NMRI, Møllegaard, Denmark, 20–22 g) were taken individually and their tails immersed in a 55°C water bath and the latency to removal measured. Thirty minutes after s.c. injection of drug or vehicle, a postdrug latency was obtained, a cutoff time of 10 sec was used. Each animal was used only once. The pre- and postdrug latencies were summarized over the 10 mice in each group and the percentage analgesia calculated with the following formula: (Postdrug latency – predrug latency/cutoff time – predrug latency) × 100.

**Materials**

Propoxy-TZTP, 3-Cl-propoxy-TZTP, xanomeline (hexyloxy-TZTP) and hexylthio-TZTP (see fig. 1) were synthesized according to the method published by Sauerberg et al. (1992), RS86 and aceclidine were synthesized at Lilly Research Laboratories. Carbachol, oxotremorine sesquifumarate, arecoline hydrobromide and pilocarpine hydrochloride (&#8734;) were purchased from Research Biochemicals International, Natick, MA.

**Results**

**In vitro receptor binding.** The IC₅₀ values for the muscarinic agonists and the thiaz diazole tetrahydropyridine analogs of arecoline propoxy-TZTP, 3-Cl-propylthio-TZTP, xanomeline and hexylthio-TZTP for inhibiting [³H]oxotremorine-M and [³H]pirenzepine are shown in table 2. All of the compounds, including the substituted TZTPs, showed high affinity in both [³H]oxotremorine-M and [³H]pirenzepine binding.

**Antinociceptive effects of muscarinic agonists.** The muscarinic agonist oxotremorine produced dose-related antinociceptive effects in each of the analgesia tests (figs. 2–5.

![Chemical structure of substituted TZTPs.](image)
and table 1). The ED$_{50}$ values for oxotremorine varied from 0.04 to 0.15 mg/kg across the tests. In addition, the muscarinic agonists arecoline, pilocarpine, aceclidine and RS86 produced antinociceptive effects in each of the analgesia tests (figs. 2–5 and table 1). In general, the order of potencies was RS86 $\gg$ pilocarpine $\gtrsim$ aceclidine $>\text{arecoline}$. In addition, the arecoline analogs propoxy-TZTP, 3-Cl-propylthio-TZTP, xanomeline and hexylthio-TZTP also produced antinociceptive effects in each of the analgesia tests. In general, the order of potencies was propoxy-TZTP $\gg$ 3-Cl-propoxy-TZTP $\gg$ xanomeline $\gg$ hexylthio-TZTP (table 1). For purposes of comparison, the opioids morphine and pethidine were also tested. In all tests, morphine was more potent than pethidine (table 1).

**Stereospecificity.** The stereospecificity of the antinociceptive effects of muscarinic agonists was evaluated by testing the isomers of aceclidine in the writhing test. As may be seen in figure 6, the antinociceptive effects of aceclidine in writhing were stereospecific: $S$-aceclidine (ED$_{50}$ = 2.5 mg/kg) was approximately twice as potent as racemic aceclidine (5.3 mg/kg), whereas $R$-aceclidine was inactive up to a dose of 30 mg/kg.

**Antagonism by scopolamine but not methscopolamine.** To determine if the effects of muscarinic agonists were mediated directly by muscarinic receptors rather than indirectly by release of endogenous enkephalins, the effects of oxotremorine (0.1 mg/kg) were determined alone and in the presence of the selective opioid antagonist naltrexone. Oxotremorine (0.1 mg/kg) administered alone inhibited acetic acid-induced writhing. Naltrexone (0.1–10 mg/kg) failed to antagonize the antinociceptive effects of oxotremorine (data not shown). At a dose of 10 mg/kg, however, methscopolamine produced a partial reversal of the antinociceptive effects of oxotremorine (fig. 8).

**Lack of antagonism by naltrexone.** To determine if the effects of muscarinic agonists were mediated directly by muscarinic receptors rather than indirectly by release of endogenous enkephalins, the effects of oxotremorine (0.1 mg/kg) were determined alone and in the presence of graded doses of the muscarinic antagonist scopolamine (fig. 7). Scopolamine (0.1–1.0 mg/kg) produced dose-related shifts to the right in the dose-response curve of oxotremorine. Scopolamine administered alone did not inhibit writhing (fig. 7).

In contrast, the quaternary analog of scopolamine, methscopolamine, which crosses the blood-brain barrier only poorly, did not antagonize the antinociceptive effects of oxotremorine (0.1 mg/kg) in the writhing test at doses of 0.1 and 1.0 mg/kg (fig. 8). At a dose of 10 mg/kg, however, methscopolamine produced a partial reversal of the antinociceptive effects of oxotremorine (fig. 8).

**Inositol phosphate hydrolysis in BHK cells cloned with the human m$_1$ muscarinic receptor.** The results are shown in table 2. Propoxy- and 3-Cl-propylthio-TZTP produced only 25 and 5%, respectively, of the maximal increase in phosphoinositide hydrolysis obtainable with carbachol,
The IC50 values and percent maximal inhibition for the muscarinic agonists oxotremorine, arecoline, pilocarpine, aceclidine and RS86 are shown in table 2 for purposes of comparison.

With IC50 values of 90 and 2000 nM, respectively, xanomeline (EC50 = 65 nM) produced a response 65% that of carbachol in the guinea pig whole ileum (table 2). Xanomeline (EC50 = 65 nM) produced a response 100% that of carbachol in the guinea pig ileum (table 2). Xanomeline (EC50 = 65 nM) produced a response 100% that of carbachol in the guinea pig ileum (table 2). The data for oxotremorine, arecoline, pilocarpine, aceclidine and RS86 are shown in table 2 for purposes of comparison.

**Guinea pig ileum.** Only propoxy-TZTP (EC50 = 75 nM) produced a response of 100% that of carbachol in the guinea pig whole ileum (table 2). Xanomeline (EC50 = 65 nM) produced a response 65% that of carbachol (table 2). 3-Cl-propylthio and hexylthio-TZTP produced responses only 27 and 10%, respectively, that of carbachol (table 2). The data for oxotremorine, arecoline, pilocarpine, aceclidine and RS86 are shown in table 2 for purposes of comparison.

**Rabbit vas deferens.** Neither propoxy-TZTP nor 3-Cl-propylthio-TZTP produced greater than a 25% inhibition of twitch height in the rabbit vas deferens at concentrations up to 30,000 nM (table 2). In contrast, xanomeline and hexylthio-TZTP both inhibited twitch height by greater than 90% with IC50 values of 0.006 and 0.001 nM, respectively (table 2). The IC50 values and percent maximal inhibition for the muscarinic agonists oxotremorine, arecoline, pilocarpine, aceclidine and RS86 are re-presented from Shannon et al. (1993) in table 2 for purposes of comparison.

**Discussion**

The present studies show that a wide range of cholinergic muscarinic agonists are potent and efficacious analgesics in mice. These data extend previous studies in the literature which show similar effects with muscarinic agonists (e.g., Ireson, 1970; Harris et al., 1969; Pedigo et al., 1975). Furthermore, we have shown that these antinociceptive effects meet the criteria for classification as a specific, receptor-mediated effect. Muscarinic agonists from several different chemical classes were tested, and each agonist produced dose-related antinociceptive effects in each of the different analgesia assays. Stereospecificity was required for producing antinociception: S-aceclidine was approximately twice as potent as racemic aceclidine, whereas R-aceclidine was inactive up to a dose more than 10-fold greater than the ED50 for S-aceclidine. Further, the antinociceptive effects of oxotremorine were antagonized in an apparently competitive manner by the specific muscarinic antagonist atropine. In addition, methscopolamine, the quaternary analog of scopalamine which crosses the blood-brain barrier only poorly, was at least 100 times less potent than scopolamine in antagonizing the effects of oxotremorine. These latter data indicate that the muscarinic receptors mediating antinociception are within the central nervous system.

Opioids are well known to affect cholinergic systems in the brain and peripheral tissues and there has been considerable interest in the potential role of acetylcholine in the analgesic mechanisms of opioids (e.g., Harris et al., 1969). Muscarinic agonists potentiate the analgesic effects of full opioid agonists in humans (Stone et al., 1961) and animals (Ireson, 1970), and those of partial agonists or mixed agonist–antagonists, such as pentaazocine (Harris et al., 1969). However, in the present study, naltrexone failed to antagonize the antinociceptive effects of oxotremorine in the writhing assay, which showed that the effects of the muscarinic agonist are not mediated by modulation of opioid receptors.

Until recently, it has not been possible to determine pharmacologically which of the muscarinic receptor subtypes mediate antinociception. The advent of functionally selective muscarinic agonists such as the substituted TZTPs (Sauerberg et al., 1992) has made it possible to investigate this question. In this study we have used xanomeline (hexyloxy-TZTP) and hexylthio-TZTP as examples of functionally selective M1 agonists (see table 1). Both of these compounds are effective in all four analgesia tests, thus they are more effective in the very stringent tail-flick test than pethidine, although their potency in the hot-plate test was relatively low (25 and 40 mg/kg, respectively). Propoxy- and 3-Cl-propylthio-TZTP are compounds with little or no M1 receptor efficacy (see table 1). They show very low levels of activity both in the rabbit vas deferens, which is an M1 receptor assay (Eltze et al., 1988, Shannon et al., 1993), and in the BHK cells.
cloned with human M₁ receptors. Both compounds are potent analgesics, approximately equivalent in potency and efficacy with morphine, and clearly more potent and efficacious than pethidine. In each of the mouse analgesia assays used, propoxy-TZTP and 3-Cl-propylthio-TZTP are between 2 and 20 times more potent than the selective M₁ agonists, xanomeline and hexylthio-TZTP. These data strongly suggest that muscarinic analgesia may not be mediated primarily by the M₁ receptor. This hypothesis is supported by the finding that two very potent and efficacious muscarinic analgesics, oxotremorine and RS86, are neither potent nor efficacious agonists in BHK cells transfected with the cloned m₁ receptor, and RS86 has very poor efficacy in the very sensitive rabbit vas deferens assay. Furthermore, both hexylthio- and 3-Cl-propylthio-TZTP lacked efficacy in the guinea pig ileum, which is largely an M₃ receptor assay (Michel and Whiting, 1988), which suggests that analgesia is not mediated by the M₃ receptor subtype. The lack of efficacy of the hexylthio-TZTP compound in the guinea pig atria, an M₂ model (Birdsall et al., 1989), would further suggest that M₂ receptors may not be involved. Further data are needed, however, to

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<th>Drug</th>
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<th>Hot-Plate</th>
<th>Grid-Shock</th>
<th>Writhing</th>
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<td>Oxotremorine</td>
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<td>RS86</td>
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<td>0.46 ± 0.01</td>
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<td>6.0 ± 2.6</td>
<td>9.9 ± 4.0</td>
<td>3.2 ± 0.01</td>
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<td>Arecoline</td>
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<td>Hexylthio-TZTP</td>
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<td>Morphine</td>
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Fig. 6. Data showing the stereospecificity of the analgesic effect of R- and S-aceclidine in the mouse writhing test. Abscissa: number of writhes; ordinate: dose of compound in milligrams per kilogram on a log scale. Points above Veh represent the effects of vehicle administration.

Fig. 7. Data showing the dose-dependent antagonism of oxotremorine by scopolamine in the writhing test. Abscissa: number of writhes; ordinate: dose of compound in milligrams per kilogram on a log scale. Points above Veh represent the effects of vehicle administration.

Fig. 8. Data showing the relative lack of antagonism by methscopolamine on analgesia evoked by 0.1 mg/kg s.c. oxotremorine. Abscissa: number of writhes; ordinate: dose of compound in milligrams per kilogram. Scop, scopolamine; Metscop, methscopolamine.
more clearly delineate which receptor subtype(s) mediate muscarinic analgesia.

The sensation and perception of pain in the mammalian central nervous system is extremely complex, and there are many levels at which muscarinic agonists could exert antinociceptive effects. Nociceptors are situated on A-delta and C fibers which pass into the anterolateral white matter in the spinal cord, enter the dorsal horns of the spinal cord via the lateral division of the dorsal roots and terminate in Rexed’s laminae I, II, III, IV and V, where the main projection neurons of the spinothalamic and spinoceccatal tracts are situated. Clearly the dorsal horn of the spinal cord, especially the substantia gelatinosa, would be a potential target area for antinociceptive compounds. Furthermore, both M1 and M2 receptors have been shown to be present in laminae II and III (Gillberg and Askmark, 1991). Although there is a lack of molecular data concerning the expression of different muscarinic receptor subtypes in the spinal cord, recent studies (Iwamoto et al., 1992; Iwamoto and Marion, 1993) have shown that the muscarinic agonist (+-cis-methylxatoloxane was effective as an antinociceptive agent after intrathecal administration to rats in both hot-plate and tail-flick assays.

The major pathway of the projection neurons is via the spinothalamic tract to the ventral posterior lateral nucleus of the thalamus (neospinotahalic) or to the posterior nuclear group and the interlaminar nuclei (paleospinotahalic). The predominant muscarinic receptor in the thalamus is the M2 receptor for which both mRNA (Buckley et al., 1988) and receptor protein have been found (Levey et al., 1991). However, protein (m1 and m2) and mRNA (m1, m2, m3, and m4) for the other subtypes also have been found in the thalamus (Buckley et al., 1988; Levey et al., 1991).

The spinothalamai tract also projects to areas in the brainstem and the mesencephalon, which may be involved in the centrifugal control of nociception. In these regions, again, the M2 receptor is the predominant subtype (Buckley et al., 1988; Levey et al., 1991; Levey, 1993). The lack of efficacy of heptane-TZTP in the isolated atria, however, suggests that M2 receptor agonism may not be the mode of action of these compounds. From the thalamus, neurons convey pain stimuli to the cerebral cortex (mainly to the post central gyrus) and the limbic system. In these areas receptor protein and mRNA have been found for the subtypes m1 to m4 (Buckley et al., 1988; Levey et al., 1991; Levey, 1993).

To summarize, although the primary effect does not seem to be M1 mediated, it is not possible from the available data to determine if the analgesic effects of muscarinic agonists are mediated by a specific subtype or by actions at more than one subtype at more than one site in the central nervous system, especially because the present study has only investigated the pharmacologically definable M1, M2, and M3 subtypes; thus, the importance of M4 or M5 subtypes cannot presently be investigated. The discovery of further compounds with different subtype selectivities or of subtype-selective antagonists would aid such studies.

In conclusion, a wide range of muscarinic agonists have been shown to be potent and efficacious analgesics in mice. The effects are mediated by central acetylcholinergic muscarinic receptors. The data obtained with some novel selective agonists suggest that an action at the M3 subtype alone does not mediate analgesia. Further work is required to elucidate the subtype or subtypes involved.

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