

Effects of Azepevole on Opioid Receptors and Endogenous Opioid Release in the Guinea Pig Ileum

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The interactions of the opioid and adrenergic systems were investigated in the guinea pig myenteric plexus longitudinal muscle preparation. Morphine and azepevole (a highly selective α_2 -adrenergic agonist) inhibit the electrically induced contractions with ED_{50} of 1.9×10^{-7} M and 3.1×10^{-6} M, respectively. The effect of morphine but not that of azepevole was competitively antagonized by naloxone. Stimulation of the preparation at 10 Hz was used to induce endogenous opioid release that was unaffected by azepevole. The authors' findings indicate that the effects of morphine and azepevole are additive, but that there is no direct interaction between the opioid and adrenergic receptors in the ileum. These observations provide some additional insight into the ability of α_2 -agonists to enhance the effects of opioids and inhalation anesthetics. (Key words: Analgesics, opioids: morphine. Receptors: opiate. Sympathetic nervous system, α_2 -agonists: azepevole.)

α_2 -ADRENERGIC AGONISTS such as clonidine and azepevole have been reported to enhance the effects of opioids and inhalational agents during anesthesia.¹⁻⁵ Additionally, recent investigations have demonstrated that α_2 -adrenergic agonists produce analgesia or anesthesia in several animal species,⁶⁻⁸ and possibly in humans.^{3,9} While the mechanisms underlying these effects have not been established, they could be explained by a decrease in neurotransmitter release at different sites in the central nervous system. The effects of α_2 -adrenergic agonists on sympathetic outflow are well documented¹⁰; however, their impact upon other neurotransmitters, such as the endogenous opioid peptides (EOP), are poorly understood.⁴ Several additional lines of evidence suggest an interaction between the opioid and adrenergic systems: 1) the analgesia induced by α_2 -adrenergic agonists is antagonized by naloxone in some, but not all, models;^{11,12} 2) cross tolerance exists between these agents and opiates;¹³ and 3) they suppress opiate withdrawal.^{12,14}

The guinea pig myenteric plexus longitudinal muscle (MPLM) preparation was used in this study because: 1) it contains both opiate¹⁵ and α_2 -adrenergic receptors;¹⁶ 2)

it possesses enkephalin and dynorphin-containing neurons;¹⁷⁻¹⁹ and 3) high-frequency electrical stimulation induces endogenous opioid release.²⁰⁻²²

The interaction between opioids and α_2 -agonists is of considerable clinical interest. The present study uses an *in vitro* preparation to characterize the interaction between azepevole (a highly specific α_2 -agonist²³) and morphine, and to determine if azepevole produces its effects by interacting directly with opiate receptors or by bringing about the release of endogenous opioid peptides.

Methods

MYENTERIC PLEXUS-LONGITUDINAL MUSCLE PREPARATION

The effects of azepevole on ileal muscle were investigated in the myenteric plexus longitudinal muscle preparation. Male albino guinea pigs (English short hair) weighing 250-300 g were decapitated and the MPLM prepared as described by Puig *et al.*²⁰ Anesthesia was not employed prior to killing because of the possible impact of an anesthetic on the interaction between azepevole and morphine. The experimental protocol was approved by the Animal Studies Committee. Strips of tissue, weighing 25-30 mg, were suspended in a 10-ml organ bath containing Krebs bicarbonate solution equilibrated at 37° C with 95% O₂-5% CO₂. The strips were placed under a resting tension of 0.3 g and were allowed to equilibrate for 60 min before starting the experiments. The tissue was stimulated with platinum ring electrodes at the top and bottom of the muscle strip. Symmetrical biphasic stimulation of 1 ms duration and supramaximal voltage (35 V) were generated by a Grass® S-88 stimulator, mixed with the aid of two Grass® stimulation isolation units (SIU5) and fed into a Crown® DC-300 audio amplifier. Strips were stimulated at a frequency of 0.1 Hz. In some experiments the tissue was also stimulated at 10 Hz as a means of eliciting a release of endogenous peptides; high-frequency stimulation was carried out for 30 s periods. Isometric contractions of the muscle were registered by means of a Grass® force transducer (Model FT03C) coupled to a Grass® polygraph recorder.

The following groups of experiments were performed: 1) dose-response curves of morphine and azepevole alone, combined or in the presence and absence of naloxone in tissues stimulated at 0.1 Hz; and 2) experiments in which the MPLM was stimulated at 10 Hz in order to elicit en-

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ogenous opioid peptide (EOP) release, in presence and absence of azepexole. In each case drugs were added to the muscle bath in a volume of 0.1 ml. For the construction of dose-response curves the tissue was exposed to each dose of the drug for 3 min (sufficient time to achieve a steady-state response) and then washed with drug-free Krebs solution prior to the addition of the next dose. In the second of the above mentioned protocols both control and azepexole-treated muscle strips were initially stimulated at 0.1 Hz (baseline stimulation) followed by five 30-s intervals at 10 Hz. The fifth period of stimulation was performed in the presence of naloxone (5×10^{-7} M), and the response was compared with that obtained during the preceding periods of stimulation.²⁰ The inhibitory response obtained after stimulation at 10 Hz was compared in the presence and absence of naloxone. The ability of naloxone to antagonize the inhibitory response induced by high-frequency stimulation (10 Hz) has been accepted as pharmacologic evidence of EOP release in this preparation.²⁰⁻²² These results were expressed as inhibitory response (IR) that was calculated by measuring the area of the electrically induced contractions 5 min before (basal response, BR) and 5 min after (poststimulation response, PSR) the stimulation at 10 Hz. The formula used for the calculations is the following:

$$IR = (BR - PSR/BR) \times 100.$$

QUANTITATION OF THE RESULTS

The potencies of morphine and azepexole under various experimental conditions were expressed as their

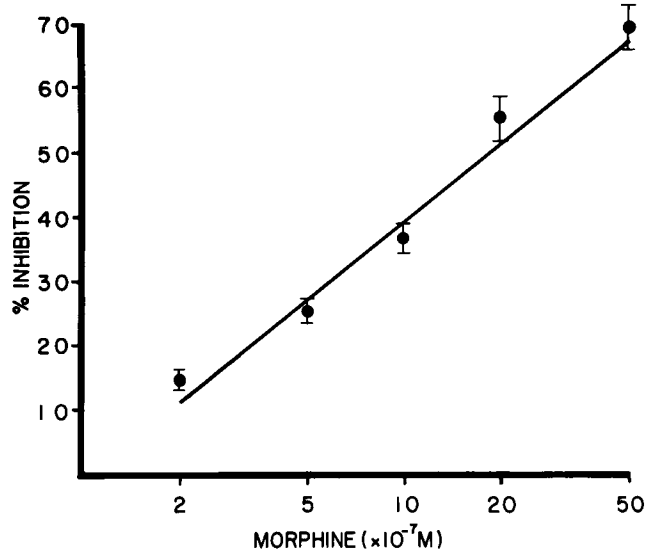


FIG. 1. Effect of morphine on electrically induced contractions of guinea pig myenteric plexus longitudinal muscle (MPLM). Each point represents the mean and SEM of the response at each concentration. The line is the least square fit of individual data points. Data were obtained from 13 muscle strips.

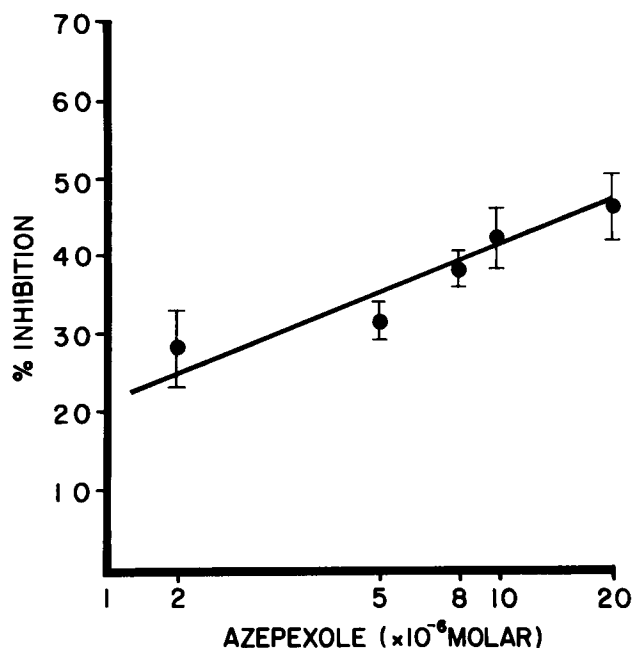


FIG. 2. Effect of azepexole on electrically induced contractions of the MPLM. Each point represents the mean and SEM of the response at each concentration. The line is the least square fit of individual data points. Data were obtained from 12 muscle strips.

ED₅₀'s. When computing these values the data from all of the corresponding log dose-response data was pooled to produce one ED₅₀ with 95% confidence limits (CL). The ED₅₀ from different treatment groups were considered to differ significantly if their confidence limits did not overlap. Maximal response of azepexole was calculated from double reciprocal plots of dose-response data. The nature of the effect of naloxone on azepexole was investigated by the use of Schild plots. All of the above procedures were performed using the methods of Tallarida and Murray.²⁴

DRUGS AND CHEMICALS

In all experiments, drug solutions were prepared daily in distilled water. The drugs used were morphine sulphate (Commons Brothers, New York, NY); naloxone hydrochloride (Sigma Chemical Co. Ltd., St. Louis, MO.); azepexole dihydrochloride (Boehringer Ingelheim, Ingelheim am Rhein, West Germany).

Results

EFFECTS OF MORPHINE AND AZEPEXOLE IN THE MPLM

Morphine and azepexole both inhibit the electrically induced contractions of the MPLM in a dose-related manner (figs. 1 and 2). The ED₅₀ of morphine was 1.9

$\times 10^{-7}$ M (CL $1.5 - 2.3 \times 10^{-7}$ M, $n = 13$), and the ED_{50} of azepexole was 3.1×10^{-6} M (CL $2.1 - 4.4 \times 10^{-6}$ M, $n = 12$). In contrast to morphine, azepexole was not able to produce complete inhibition of the electrically induced muscle contractions. A double reciprocal analysis of the azepexole data revealed a maximal inhibitory effect of 60%. As a means of investigating the mutual interactions of these two agonists, dose-response curves to morphine were performed in the presence of increasing concentrations of azepexole ($2, 5, \text{ and } 8 \times 10^{-6}$ M). In these experiments each dose of morphine produced the same response in the presence or absence of azepexole, indicating that the effects of both agonists were additive (table 1 and fig. 3).

EFFECTS OF NALOXONE ON THE ACTION OF AZEPEXOLE IN THE MPLM

We have previously shown that naloxone, in the concentration range of $1 \text{ to } 30 \times 10^{-9}$ M, competitively antagonized the effects of morphine in this preparation.²⁵ In the present study, naloxone did not competitively antagonize the effects of azepexole. At a concentration of 3×10^{-9} M, naloxone produced an increase (50%) in the ED_{50} of azepexole; however, further increments in the concentration of naloxone failed to produce any additional changes (table 2). The slope of the dose-response curve to azepexole alone was 22.2 ± 4.1 and in the presence of naloxone (1×10^{-7} M) was 27.2 ± 1.5 . A Schild plot was constructed using naloxone concentrations of 1×10^{-9} M to 1×10^{-7} M. The slope of the resulting plot was -0.28 ± 0.14 which indicates that the observed antagonism was not competitive in nature, thus PA_2 could not be calculated under these experimental conditions.

The effects of azepexole on EOP release (elicited by stimulation at 10 Hz) were determined in a series of five experiments. In each experiment one strip of muscle was constantly exposed to azepexole at a concentration of 2×10^{-6} M. Another strip of muscle from the same animal served as a control. In both cases the tissues were stimu-

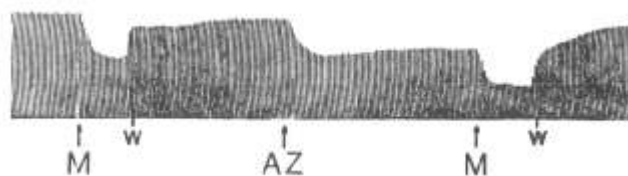


FIG. 3. Effect of morphine (M) in the absence and presence of azepexole (AZ). The MPLM was stimulated at 0.1 Hz. Addition of 1×10^{-7} M morphine produced a 38% and 42% inhibition, respectively, before and after azepexole (3×10^{-6} M). W indicates washing of the preparation with drug-free Krebs bicarbonate solution.

lated at 10 Hz five consecutive times. The final stimulation of each was performed in the presence of naloxone (5×10^{-7} M). Our results indicate that the inhibitory response (IR) observed after 10-Hz stimulation was unaltered by the presence of azepexole. Thus, in strips treated with azepexole ($n = 5$) the IR was 40.1 ± 9.0 (mean \pm SE) while in the control group it was 40.2 ± 10.8 . Furthermore, naloxone reduced the IR to 22.1 ± 4.7 and 18.6 ± 4.2 in control and azepexole-treated preparations, respectively.

Discussion

Azepexole is an α_2 -adrenergic agonist that has a mode of action similar to clonidine.²³ Both compounds have been found to reduce anesthetic requirements,^{2,5} but the mechanism of this effect is not well established. Several hypotheses can be proposed to explain this observed interaction: 1) release of endogenous opioid peptides and/or direct interaction with opioid receptors; 2) alterations in other neurotransmitter systems that could produce analgesia; and 3) decreased sympathetic outflow that could mask the physiologic responses to pain during anesthesia.

Data from *in vivo* studies argue against azepexole masking the response to painful stimuli,^{26,27} and the present study demonstrates that azepexole does not directly in-

TABLE 1. Effect of Azepexole on the ED_{50} of Morphine

	Azepexole ($\times 10^{-6}$ M)			
	0	2	5	8
ED_{50} MS ($\times 10^{-7}$ M)	1.9	1.1	1.7	2
CL ($\times 10^{-7}$ M)	1.5-2.3	0.7-1.8	1.3-2.3	1.7-2.4
Number of animals	9	6	6	6

None of the values are significantly different from the ED_{50} of morphine alone.

MS = morphine sulphate. CL = 95% confidence limits.

TABLE 2. Effects of Naloxone on the Potency of Azepexole

Naloxone ($\times 10^{-9}$ M)	ED_{50} Azepexole ($\times 10^{-6}$ M)
0	3.1 (2.1-4.4)
1	3.8 (3.1-4.7)
3	5.3 (4.2-6.7)
30	5.1 (4.4-5.8)*
100	5.5 (4.5-6.6)*

Values in parentheses are the 95% confidence limits of the ED_{50} of azepexole.

* Significant difference ($P < 0.05$) when compared with the corresponding value in the absence of naloxone. Each value is the result of six or more determinations.

teract with opioid receptors nor does it alter EOP release in the guinea pig ileum in this preparation.

In this preparation, morphine produces a dose-dependent inhibition of electrically induced muscle contractions. This effect is competitively antagonized by naloxone and presumably reflects an interaction with μ -opioid receptors; the PA_2 previously obtained in our laboratory²⁵ in this tissue is similar to the values observed by other investigators for μ -opioid receptors.²⁸ Azepevole also produces an inhibitory response in the MPLM.²⁹ However, naloxone cannot be considered to be a competitive antagonist of azepevole because: 1) the effect of naloxone was not dose-related (table 2); 2) the resulting dose-response curves were not parallel; and 3) the slope of the Schild plot differed significantly from -1 , a further indication that a competitive interaction did not occur. Moreover, similar conclusions were drawn using clonidine in studies performed with tissues obtained from morphine dependent animals.³⁰ In a previous study, yohimbine competitively antagonized the effects of azepevole in this tissue with a PA_2 that is indicative of an interaction with an α_2 receptor.²⁹

Azepevole did not have an apparent effect on EOP release, as indirectly measured by stimulation of the MPLM preparation at 10 Hz, since the inhibitory response (see Methods) observed after high-frequency stimulation and its reversal by naloxone were unaltered. These observations indicate that the effects of azepevole are not mediated either by direct interaction with μ -opioid receptors nor by release of EOP in this preparation.

In several *in vivo* systems, α_2 -adrenergic agonists appear to enhance the effects of opiates,¹⁻⁴ but the interpretation of previous studies is made difficult by the complexity of the systems used. Our findings in an *in vitro* preparation of an additive interaction between azepevole and morphine are consistent with these earlier studies. However, no direct interaction between the opioid and adrenergic systems could be demonstrated. The effects of α_2 -agonists on anesthetic requirements *in vivo* might be explained on the basis of the actions of these agents on other neurochemical systems represented within the guinea pig ileum.

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