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BRIEF COMMUNICATION

Modification of *Alpha*-Adrenoceptor Agonist Antinociceptive Activity by Nisoxetine: A Selective Inhibitor of Noradrenergic Uptake

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HYNES, M. D. AND J. K. HENDERSON. *Modification of* alpha-*adrenoceptor agonist antinociceptive activity by nisoxetine: A selective inhibitor of noradrenergic uptake.* PHARMACOL BIOCHEM BEHAV **20**(3) 463–466, 1984.—The *alpha*-adrenoceptor agonists, clonidine and xylazine, produced a dose-dependent antinociceptive effect in the mouse writhing assay as does morphine. Fluoxetine, a highly-specific inhibitor of serotonin uptake, enhanced the antinociceptive effect of morphine in this test but not that of clonidine or xylazine. In contrast, nisoxetine, a selective inhibitor of noradrenergic uptake, significantly potentiated the antinociceptive activity of morphine, clonidine, and xylazine. These findings strengthen the evidence for an involvement of a noradrenergic mechanism in the antinociceptive effects of *alpha*-adrenoceptor agonists.

Fluoxetine Xylazine Nisoxetine Antinociception Morphine Serotonin Clonidine Noradrenaline

THE antinociceptive activity of clonidine, an *alpha*-adrenoceptor agonist with clinically useful antihypertensive activity, has been demonstrated in a variety of testing procedures to measure pain in laboratory animals [5, 6, 14, 27]. Xylazine, an *alpha*-adrenoceptor agonist closely resembling clonidine in structure, is employed as an analgesic in veterinary medicine [3]. The exact mechanism by which clonidine and xylazine induce an antinociceptive effect is not known. Since the antinociceptive effects of these *alpha*-adrenoceptor agonists are not blocked by narcotic antagonist [7, 20, 22] and they do not exhibit cross-tolerance to morphine [29], an interaction with opioid receptrs has been discounted.

Much evidence has accumulated to suggest a role for the serotoninergic neurons in analgesia [26]. Morphine analgesia, for example, is potentiated by the enhancement of serotoninergic function [13, 18, 20, 28, 32] and antagonized by treatments that deplete serotonin [10, 11, 30, 33, 37]. Despite all that is known about the role of serotonin in morphine analgesia, little is known of its involvement in non-opioid analgesia, such as the type mediated by clonidine-like *alpha*-adrenoceptor agonists. Experiments were undertaken to ascertain the effects of enhancing serotonin function on the antinociceptive activity of clonidine and xylazine in mice. The highly-specific inhibitor of serotonin uptake, fluoxetine [8, 35], which potentiates morphine analgesia [15, 20, 31], was selected for the purpose of enhancing serotoninergic function in this study.

Since a role of *alpha*-adrenoceptors in the antinociceptive activity of clonidine and xylazine has been well-documented [5, 7, 12, 14, 21, 22, 23], the second goal was to investigate the effect of enhancing noradrenergic function on the analgesic activity of clonidine and xylazine. Nisoxetine, a selective inhibitor of noradrenaline uptake [9, 34, 36], was employed to enhance noradrenergic function.

METHOD

Mouse Writhing Assay

The mouse writhing response was defined as a contraction of the abdominal musculature followed by the extension of the hind limbs. Acetic acid administered intraperitoneally (10 ml/kg) at a concentration of 0.6 percent was used to induce the writhing response. Five Cox Standard albino mice (Laboratory Supply Co., Indianapolis, IN), weighing 20 to 22 grams after being fasted overnight, were observed simultaneously for the occurrence of the writhing response. Each mouse was used only once. The observation period was ten minutes and started five minutes after the administration of acetic acid. Inhibition of mouse writhing was calculated from

TABLE 1

DIFFERENTIAL EFFECTS OF FLUOXETINE AND NISOXETINE ON THE ANTINOCICEPTIVE ACTIVITY OF CLONIDINE, XYLAZINE, AND MORPHINE

	Inhibition of Mouse Writhing* ED_{50} (95 Percent Confidence Limits)		
Pre-Treatment†	Morphine	Clonidine	Xylazine
Saline	0.80 (0.65–0.98)	0.012 (0.008–0.017)	0.56 (0.47–0.67)
Fluoxetine	0.23 (0.18–0.30)	0.008 (0.006–0.010)	0.41 (0.34–0.50)
Nisoxetine	0.30 (0.17–0.50)	0.005 (0.004–0.007)	0.21 (0.13–0.32)

*Mouse writhing ED_{50} values are expressed in mg/kg and were calculated by the use of the regression line in reverse [4]. Given in the parentheses is the range of values for the 95 percent confidence limits. Control mice exhibited an average of 260 writhes in the ten-minute observation period.

†All drugs were administered by the subcutaneous route 30 minutes prior to testing with the exception of clonidine which was given 15 minutes before the measurement of writhing. Fluoxetine and nisoxetine were administered in a fixed dose of 20 mg/kg.

the total number of writhes in the control and drug treated groups according to the following formula:

$$\text{Percent Inhibition} = 100 - \frac{\text{Experimental Group} \times 100}{\text{Control Group}}$$

On the average, control mice exhibited 225 to 300 writhes in the ten-minute observation period. The dose required to reduce the frequency of writhing by 50 percent was defined as the ED_{50} and was computed by "The Use of the Regression Line in Reverse" [4].

Drugs

All the drugs employed in these experiments were dissolved in water and administered subcutaneously. Clonidine hydrochloride was a gift from Boehringer-Ingelheim; xylazine was a gift from Bayer AG; and morphine sulfate was donated by Eli Lilly and Company. The fluoxetine and nisoxetine were prepared by the Lilly Research Laboratories.

RESULTS

The effect of fluoxetine on the antinociceptive activity of morphine, clonidine, and xylazine in the mouse writhing assay is shown in Table 1. Fluoxetine, as previously reported [13], potentiated the analgesic effect of morphine in the mouse writhing test. Administration of increasing doses of morphine alone resulted in an ED_{50} value of 0.80 mg/kg. Following pretreatment with 20 mg/kg of fluoxetine, there was a parallel shift in the morphine dose-response curve. In the presence of fluoxetine, the morphine ED_{50} value for writhing inhibition was decreased to 0.23 mg/kg. Mouse writhing was not inhibited by the adminisration of fluoxetine alone.

Clonidine was found to be a potent inhibitor of mouse writhing (Table 1). An ED_{50} value of 0.012 mg/kg was calculated for clonidine. Administration of fluoxetine at a 20 mg/kg dose failed to alter the dose-response curve for clonidine. The combination of fluoxetine with clonidine yielded an ED_{50} value of 0.008 mg/kg which was not significantly different from the ED_{50} of clonidine alone.

Similar results were obtained with the *alpha*-adrenoceptor agonist, xylazine (Table 1). Xylazine produced a dose-dependent inhibition of mouse writhing yielding an ED_{50} value of 0.56 mg/kg. Simultaneous administration of fluoxetine, 20 mg/kg, failed to alter significantly the ED_{50} for xylazine.

The effect of nisoxetine on the antinociceptive activity of morphine, clonidine, and xylazine is given in Table 1. Nisoxetine, 20 mg/kg, did not produce a significant inhibition of mouse writhing when administered alone. The inhibition of writhing produced by morphine was potentiated significantly by this dose of nisoxetine as evidenced by a parallel shift to the left of the dose-response curve.

Administration of nisoxetine significantly shifted the dose-response curve for clonidine to the left in a parallel manner. The ED_{50} value of 0.005 mg/kg for the combination of clonidine plus nisoxetine was lower than that for clonidine alone, as may be seen in Table 1.

The dose-response curve for xylazine-induced inhibition of mouse writhing was also shifted in a parallel manner to the left by nisoxetine. When xylazine was administered simultaneously with 20 mg/kg of nisoxetine, the ED_{50} for writhing was 0.21 mg/kg which is significantly lower than that for xylazine alone, 0.56 mg/kg.

DISCUSSION

Fluoxetine, a specific inhibitor of serotonin uptake [3,35], enhanced the analgesic effect of morphine in the mouse writhing test. Potentiation of the antinociceptive effects of morphine in this assay was made apparent by a parallel shift in the dose-response curve and a reduction in the ED_{50} value following pretreatment with fluoxetine. These results are consistent with previous reports which show that fluoxetine potentiates the effects of morphine in the writhing [13], foot shock [20], hot plate [31], and tail flick [15] tests. These results strengthen the view that enhancement of serotoninergic function potentiates the analgesic effect of morphine.

Selective inhibition of norepinephrine uptake by nisoxetine [9, 34, 36] significantly increased the analgesic activity of morphine in the mouse writhing assay. Similarly, amitriptyline and nortriptyline, which inhibit norepinephrine uptake but also effect serotoninergic neurotransmission, have been found to potentiate morphine analgesia [17,19]. However, there are other studies in which these compounds

did not effect the analgesic activity of morphine [16]. The results with nisoxetine, a highly-selective noradrenergic uptake inhibitor, reported herein strengthens the concept that noradrenergic mechanisms are involved in morphine analgesia.

The *alpha*-adrenoceptor agonists, clonidine and xylazine, produced a dose-dependent inhibition of mouse writhing. The antinociceptive effects of these compounds have been previously demonstrated in the writhing assay [1, 2, 6, 12] and a variety of other tests for analgesic activity [2, 5, 6, 7, 14, 24, 25, 27]. However, fluoxetine did not potentiate the antinociceptive effects of these *alpha*-adrenoceptor agonists, which is evidenced by the similar ED_{50} values in the presence and absence of fluoxetine. These results are in marked contrast to those achieved with nisoxetine. The clonidine and xylazine dose-response curves for writhing inhibition were significantly shifted to the left in a parallel manner by the administration of nisoxetine. These results suggest a role for noradrenergic mechanism, but not serotoninergic, in the antinociceptive activity of *alpha*-adrenoceptor agonists. This conclusion is consistent with a number of published reports where less selective inhibitors of uptake were utilized. The antinociceptive effect of clonidine is blocked by the *alpha*-adrenoceptor antagonists phenoxybenzamine [38] and yohimbine [1, 6, 12, 22], but not by the serotonin antagonist metergoline [38]. The role of a noradrenergic mechanism in the antinociceptive effects of *alpha*-adrenoceptor agonists is also supported by the fact that there is a significant correlation between the relative potency of a series of *alpha*-adrenoceptor agonists for inhibiting writhing and their affinity for *alpha*-adrenoceptors [12].

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