Pharmacology Biochemistry & Behavior, Vol. 12, pp. 573–575. Printed in the U.S.A.

Effectiveness of Lofexidine in Blocking Morphine-Withdrawal Signs in the Rat

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Received 10 September 1979

SHEARMAN, G. T., H. LAL AND R. C. URSILLO. *Effectiveness of lofexidine in blocking morphine-withdrawal signs in the rat.* PHARMAC. BIOCHEM. BEHAV. 12(4) 573–575, 1980.—Discontinuation of chronic morphine infusion in rats resulted in the reliable occurrence of withdrawal body shakes. This sign of narcotic withdrawal was dose-dependently reduced by lofexidine (0.04–0.64 mg/kg) and clonidine (0.01–0.16 mg/kg). As with clonidine, the activity of lofexidine was not prevented by naloxone (5 mg/kg). In addition, diarrhea induced by naloxone (5 mg/kg) in morphine dependent rats was also prevented by lofexidine or clonidine pretreatment. These data suggest that lofexidine, like clonidine, may reduce the narcotic withdrawal syndrome in humans.

Morphine withdrawal Body shakes Diarrhea Lofexidine Clonidine

RECENTLY, clonidine's efficacy in reducing certain signs of narcotic withdrawal in laboratory animals [3, 15–17] and in reducing many signs and symptoms of opiate withdrawal in narcotic addicts [5,18] was reported. (For other psychopharmacological actions, see [13]). These actions of clonidine are not prevented or reversed by the narcotic antagonist, naloxone [3,18], suggesting a mechanism of action unlike that of narcotics in this respect.

Lofexidine, 2-[α-(2,6-dichlorophenoxy)-ethyl]-Δ2-imidazoline, a structural analogue of clonidine (Fig. 1) was found to possess a high affinity for [H^3]-clonidine binding sites in rat cerebral cortex [8] as well as antihypertensive activity in experimental animals and human patients [2]. Like clonidine, lofexidine inhibits castor-oil induced diarrhea in the rat suggesting another clinical use as an antidiarrheal [11]. Because of its similarity to clonidine in terms of structure and activity, lofexidine was studied for its effectiveness in blocking morphine-withdrawal signs in the rat. We now report that lofexidine compares well with clonidine in reducing morphine-withdrawal signs in the rat.

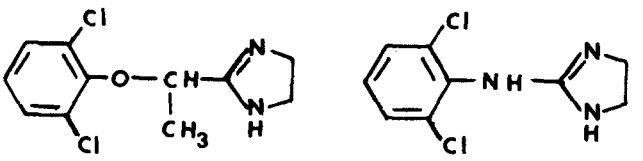

FIG. 1. A structural comparison of lofexidine with clonidine.

METHOD

Male hooded rats of the Long-Evans strain, weighing between 300–350 g, were implanted with an indwelling jugular catheter as described earlier [12,14]. Following surgery, the rats were housed individually in Plexiglas chambers. The catheter was connected to an external injection system consisting of a Harvard Apparatus Compact Infusion-Pump (Model 975). Room lights were turned on between 8 a.m. and 8 p.m. Food and water were continuously available except during the observation of withdrawal signs when food cups were removed from the chambers.

The rats were made physically dependent by continuously infusing a morphine sulfate solution in gradually increasing concentrations until a maintenance dose of 100 mg/kg/day was reached in five days. This dose continued to be infused for an additional four days prior to withdrawal. In order to precipitate withdrawal, the infusion pumpts were turned off. Eight hours after the pumps were turned off, the rats were observed for occurrence of withdrawal body shakes for a period of 30 min. Body shakes were defined as violent shaking movements of the head and/or body of the rat which resemble the action of an animal that has been drenched with water [4]. Following this observation, the animals were injected intraperiteonally with either lofexidine

TABLE 1

EFFECTIVENESS OF LOFEXIDINE AND CLONIDINE IN BLOCKING MORPHINE WITHDRAWAL BODY SHAKES IN THE RAT

Drug*	Dose† (mg/kg)	Morphine withdrawal body shakes % pretreatment [mean ± SE; (N)]‡ Time after drug injection 30 min	2 hr
Saline	—	110 ± 10 (64)	79 ± 24 (3)
Naloxone§ + Saline	5.00	105 ± 15 (8)	—
Lofexidine	0.04	72 ± 16 (5)¶	95 ± 13 (4)
	0.16	38 ± 9 (5)¶	77 ± 25 (5)
	0.64	10 ± 4 (7)¶	26 ± 8 (7)¶
Naloxone§ + Lofexidine	5.00 0.64	15 ± 7 (5)¶	31 ± 12 (5)¶
Clonidine	0.01	44 ± 9 (10)¶	—
	0.04	22 ± 8 (6)¶	—
	0.16	7 ± 5 (11)¶	7 ± 4 (5)¶
Naloxone§ + Clonidine	5.00 0.16	12 ± 4 (11)¶	—

*All drugs were injected IP in a volume of 1 ml/kg.

†Drug doses are expressed in terms of the hydrochloride salts.

‡Number in ( ) is the number of rats tested. A mean of 26 shakes was recorded during pre-drug observations.

§Animals were injected with naloxone 30 min before saline, lofexidine or clonidine.

¶Significantly different ($p<0.05$) form pre-drug shakes; Students' Paired T test.

TABLE 2

EFFECTIVENESS OF LOFEXIDINE AND CLONIDINE IN BLOCKING NALOXONE-INDUCED DIARRHEA IN MORPHINE DEPENDENT RATS

Drug*	Dose† (mg/kg)	N‡	% of rats exhibiting diarrhea Time after naloxone (min)§ 30	60	120
Saline	—	5	100	100	100
Lofexidine	0.64	5	0¶	0¶	0¶
Clonidine	0.16	5	0¶	0¶	0¶

*All drugs were injected IP in a volume of 1 ml/kg.

†Drug doses are expressed in terms of the hydrochloride salts.

‡Number of rats tested.

§Naloxone (5 mg/kg) was injected 30 min after injection of the test drug.

¶Significantly different ($p<0.05$) from saline treatment; Chi Square Analysis.

or clonidine. Thirty minutes and two hours after these injections, the rats were again observed for a period of 30 min for the occurrence of withdrawal body shakes. In other rats, naloxone was administered at the beginning of the first observation period. The effect of drug treatments on the occurrence of withdrawal body shakes was analyzed by converting postdrug shakes into a percentage of the mean predrug shakes (post-treatment shakes/mean pretreatment shakes $\times 100$). In order to measure antidiarrheal activity, lofexidine or clonidine was administered 30 min after the infusion pumps were turned off. Thirty minutes following these injections, naloxone was administered. Every 30 min, for two hours after the naloxone injection, the floor underneath each cage was examined for the presence or absence of diarrhea. Diarrhea was defined as watery and unformed stools splashed on the tray, as opposed to the normal fecal excretion that consists of well-formed boluses firm and fairly dry. The ability of lofexidine and clonidine to prevent the naloxone-induced diarrhea was measured by comparing the percentage of rats exhibiting diarrhea after these pretreatments with the percentage of rats exhibiting diarrhea after saline pretreatment.

RESULTS

It was previously established that discontinuation of chronic saline infusion into rats does not result in withdrawal body shakes, whereas the discontinuation of continuous morphine infusion results in a large number of body shakes characteristic of narcotic withdrawal [6,10]. These shakes were previously shown to be blocked by an acute injection of morphine (ED_{50}, 14 mg/kg) [6,10].

Data summarized in Table 1 show that both lofexidine and clonidine produced a dose-dependent reduction of the morphine withdrawal body shakes. The ED_{50}'s of these drugs were 0.10 mg/kg and 0.02 mg/kg, respectively. Administration of the narcotic antagonist, naloxone (5 mg/kg), did not cause a further increase in the occurrence of body shakes of rats already withdrawn from morphine [7]. Whereas naloxone (5 mg/kg) pretreatment decreased the reduction in body shakes produced by morphine [10], this pretreatment did not alter the reduction in body shakes produced by lofexidine or clonidine (Table 1).

Data summarized in Table 2 show that both lofexidine and clonidine prevented naloxone-induced diarrhea in the morphine dependent rats at doses which were about equieffective in blocking withdrawal body shakes (Table 1).

DISCUSSION

Discontinuation of chronic morphine infusion resulted in the reliable occurrence of withdrawal body shakes, a well-recognized sign of narcotic withdrawal in the rat [4]. Such shakes are known to be dose-dependently reduced by narcotics [4,10], neuroleptics [6, 7, 9, 10] and the imidazoline derivative, clonidine [3, 15–17]. The present experiment demonstrates that lofexidine, a new antihypertensive agent, also produces a dose-dependent reduction of morphine withdrawal body shakes. In this regard, both clonidine and lofexidine were much more potent than morphine. It was previously reported that the reduction of morphine withdrawal body shakes produced by clonidine was not prevented by the narcotic antagonist, naloxone [3]. Similarly, the lofexidine-induced reduction of morphine withdrawal body shakes was not prevented by naloxone pretreatment.

The mechanism by which either clonidine or lofexidine reduced the morphine-withdrawal syndrome is not known. It was recently reported that a major nerve tract containing endorphine neurons originates from the arcuate nucleus and

terminates in locus coeruleus [19] suggesting an endorphinergic influence on locus coeruleus functions. It is also known that in the locus coeruleus made tolerant to the depressant effects of morphine, naloxone causes precipitation of hyperactivity. This hyperactivity is blocked by clondine [1]. Based upon these observations, one may suggest that clondine-like drugs reduce the opiate withdrawal syndrome by modifying endorphinergic influences on locus coeruleus. Since lofexidine is equipotent to clonidine in inhibiting (^{3}H)-clonidine binding to rat brain *in vitro* [8], it may be assumed that the mechanisms of the two compounds are similar. Additionally, as with clonidine, lack of naloxone antagonism of lofexidine effects suggest that the mechanism of action of lofexidine is independent of any direct action on opioid-sensitive sites.

Diarrhea is another well-recognized sign of narcotic withdrawal in the rat [4]. It was previously reported that clonidine blocked naloxone-induced diarrhea in morphine-dependent rats [15]. Like clonidine, the present experiment demonstrates that lofexidine also blocks naloxone-induced diarrhea in morphine-dependent rats. Recently, we found that both clonidine and lofexidine produced a dose-dependent inhibition of castor oil induced diarrhea in the rat [11]. This action was neither prevented nor reversed by naloxone suggesting a mechanism of action unlike that of narcotics. The mechanism by which clondine and lofexidine inhibit naloxone-induced diarrhea in morphine-dependent rats however remains to be determined.

Blockade of narcotic withdrawal in laboratory animals has predictive value for those drugs which might be effective in clinical situations [4]. Based upon the reports that clonidine reduced narcotic withdrawal signs in laboratory animals [3, 15–17] this drug was subsequently found to alleviate narcotic withdrawal in humans [5,18]. The blockade of morphine withdrawal signs in the rat by lofexidine suggests that this new drug may also be effective in reducing narcotic withdrawal symptoms in humans.

ACKNOWLEDGEMENTS

The authors are grateful to Merrell Research Center for financial support and the supply of lofexidine. Clonidine hydrochloride and naloxone hydrochloride were gifts of Boehringer Ingelheim Ltd. and Endo Laboratories, respectively. The technical assistance of Linda Noel and Ayla Sen is greatly appreciated.

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