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EFFECTS OF CALCIUM CHANNEL ANTAGONISTS ON THE REINFORCING PROPERTIES OF MORPHINE, ETHANOL AND COCAINE AS MEASURED BY PLACE CONDITIONING

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Morphine, ethanol and cocaine were examined in place conditioning paradigm. After initial preferences were determined, animals were conditioned with morphine (5 mg/kg), ethanol (1 g/kg) and cocaine (5 mg/kg) alone or combinations of these drugs plus some calcium antagonists: nifedipine (5 and 10 mg/kg) and verapamil (5 and 10 mg/kg). Nifedipine prevented the ability of morphine and cocaine, but not of ethanol, to produce a place preference. Our results suggest that substances which can influence calcium distribution are involved in the rewarding actions of some drugs — morphine and cocaine. Dihydropyridine, a calcium channel antagonists might be clinically useful for the treatment of morphine and cocaine abuse.

Key words: place conditioning, morphine, ethanol, cocaine, nifedipine, verapamil, reward, calcium channel blockers.

INTRODUCTION

Calcium channel blockers inhibit calcium channels or calcium entry into cardiac and smooth muscle cells. They can be classified according to their chemical structure into the dihydropyridine (nifedipine, nimodipine, nicardipine), phenylalkylamine (verapamil) and benzothiazepine (diltiazem) derivatives (1).

Four distinctive categories of calcium channels have been identified: (L-, T-, N- and P-type) with the L-type having the greatest effect upon channels located on neuronal cell bodies (2).

Pharmacological studies have demonstrated that modulation of Ca^{2+} influx through L-type of calcium channels modifies the antinociceptive, respiratory and behavioural actions of several opioid agonists. It has been shown that calcium channels inhibitors suppress the morphine-withdrawal

syndrome in rats and modulate the analgesic action of morphine and of several κ -receptor agonists (3, 4).

In this study using the conditioned place preference (CPP) paradigm we investigated the rewarding properties of morphine, ethanol and cocaine. In the place conditioning, a drug treatment is paired with the external neutral stimuli of one environment. The CPP paradigm utilises the phenomenon of secondary conditioning in which a neutral stimulus that has been paired with a reward acquires the ability to serve as a reward itself (5). Moreover we measured the effects of two calcium inhibitors — nifedipine and verapamil on acquisition of the conditioned place preference induced by morphine, ethanol and cocaine to find out if there is a relation between the concentration of calcium ions and the rewarding properties of drugs of abuse.

MATERIALS AND METHODS

Apparatus

The CPP apparatus consisted of 4 rectangular wooden boxes that had three different compartments separated by removable guillotine doors. The two end compartments measured 25 × 35 cm while the middle compartment — 10 × 10 cm. One end compartment has white walls and a mesh floor. The other had black walls and a metal grid floor. The middle compartment had grey walls and a solid wood floor. The testing boxes were housed in a soundproof room with white noise and constant light provided by a 40 W lamp placed above the compartment.

Procedure

There were 3 phases of behavioural testing: pre-test, conditioning and test. During the pre-test each rat was placed in the central grey area in an undrugged state and allowed to explore the 3 compartments for 15 min. The time spent by each rat in the two large compartments of the box was recorded manually on the second day. The side on which the animal spent the least time on the pre-test day was considered to be the non-preferred side. Conditioning consisted of 3 days, exceptionally — when ethanol was injected — 10 days. During this phase, the rats were exposed for 30 min to the preferred compartment after administration of the vehicle and after an interval of 4 h — to the non-preferred compartment after administration of the drug. During the third phase, the guillotine doors were removed and the time spent by each rat in the two distinctive compartments was recorded during 15 min of observation. Animals received no drugs during test day.

Animals

Experimental naive male rats Wistar weighing between 200 and 250 g were housed 6 per cage with food and water freely available. The animals were maintained under a 12 h light-dark cycle and were adapted to laboratory conditions for at least one week.

Drugs

Drugs and respective vehicle for associated control groups were administered intraperitoneally (ip) in a volume of 0,5 ml/100 g body weight. The drugs used were: morphine HCl (Polfa), ethanol (20% v/v), cocaine HCl (Sigma), nifedipine (Polfa) and verapamil HCl (Knoll). Nifedipine and verapamil were administered 15 min before morphine, ethanol or cocaine injection. Morphine, ethanol and cocaine were given immediately before each of the conditioning sessions.

Statistics

The significance of differences in second between the time spent in the vehicle- and drug-paired non-preferred compartment in the post-conditioning test was evaluated by Student t-test. The confidence limit of $p < 0.05$ was considered as statistically significant.

RESULTS

Morphine

As shown in *Fig. 1 and 2*, morphine (5 mg/kg) elicited statistically significant place preference. Pairing of nifedipine (5 and 10 mg/kg) prevented morphine-induced place preference ($p < 0.05$) (*Fig. 1*). Nifedipine administered at the dose 2.5 mg/kg was without effect. Nifedipine at both active doses also increased the time spent in the non-preferred compartment ($p < 0.05$ and $p < 0.01$, *Fig. 1*). Pretreatment with verapamil (5 and 10 mg/kg) did not prevent acquisition of the conditioned place preference induced by morphine. Verapamil alone at both doses used increased the time spent in the non-preferred compartment ($p < 0.05$ and $p < 0.01$, *Fig. 2*).

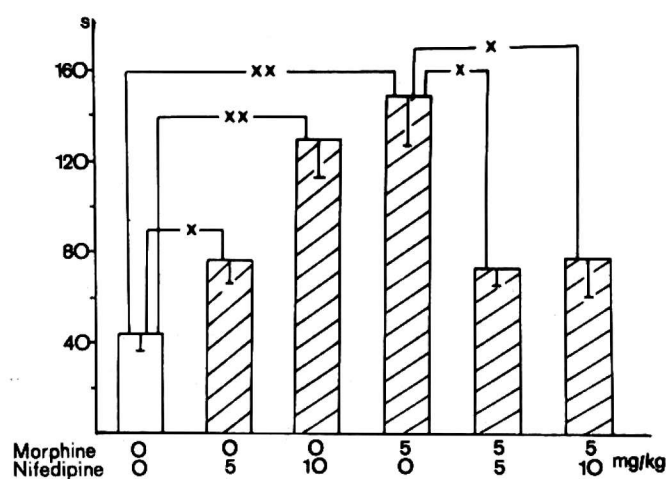


Fig. 1. Effects of nifedipine on acquisition of the conditioned place preference induced by morphine. Each bar represents means \pm SEM of time (in s) spent in the non-preferred compartment after conditioning. * $p < 0.05$; ** $p < 0.01$.

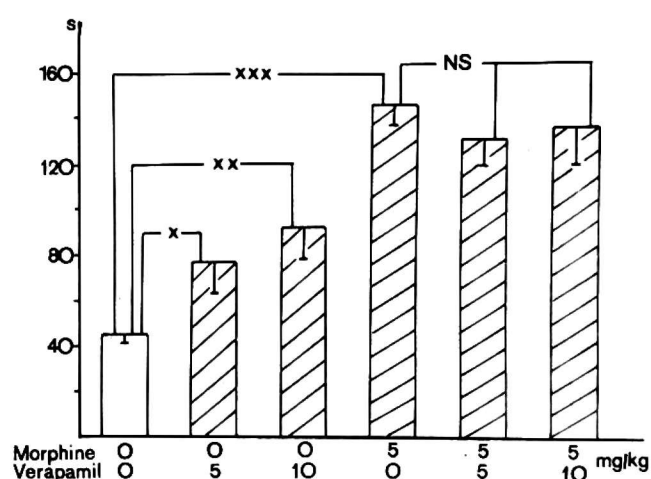


Fig. 2. Effects of verapamil on acquisition of the conditioned place preference induced by morphine. Each bar represents means \pm SEM of time (in s) spent in the non-preferred compartment after conditioning. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Ethanol

Ethanol at the dose 1 g/kg elicited significant place preference ($p < 0.01$, Fig. 3). Pretreatment with nifedipine (5 mg/kg) did not alter ethanol-induced place preference conditioning.

Cocaine

As shown in Fig. 3, cocaine at the dose 5 mg/kg increased the time spent in the non-preferred compartment as compared with control group ($p < 0.05$). Pairing of nifedipine (5 mg/kg) completely prevented acquisition of the conditioned place preference induced by cocaine ($p < 0.01$).

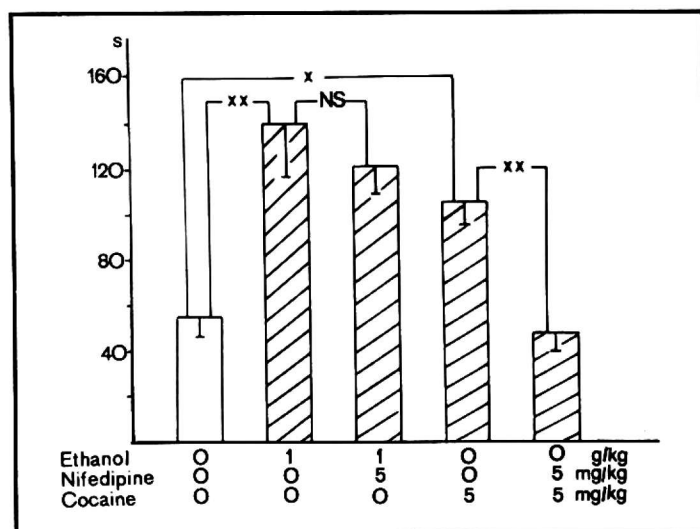


Fig. 3. Effects of nifedipine on acquisition of the conditioned place preference induced by ethanol and cocaine. Each bar represents means \pm SEM of time (in s) spent in the non-preferred compartment after conditioning. * $p < 0.05$; ** $p < 0.01$.

DISCUSSION

The present results show that nifedipine given before each of the drugs injections can influence the rewarding effects of some positive reinforcers — morphine and cocaine, but not of ethanol, as measured using the CPP procedure. Recently it has been shown that calcium channel inhibitors suppress most of the signs of morphine withdrawal (body weight loss, diarrhoea, “wet dog shakes”) and enhance the analgesic effects of opioids. Physical dependence on morphine is associated with a large increase in the content of Ca^{2+} in the brain, which returned towards normal during precipitated withdrawal. If morphine is administered at the time in which the calcium channels are blocked, the physical dependence would not develop (4, 6). It suggests that calcium channel blockers should be tested in the treatment of opioid addicts.

A great deal of evidence suggests, that mesolimbic dopamine system is involved in the reward produced by opioid receptors agonists and other positive reinforcers (7, 8). Calcium channel inhibitors can act to attenuate dopamine release at the synapse. They are also capable of inhibiting morphine- and cocaine-induced dopamine release in the mesolimbic system (9). However,

our results suggest that while dihydropyridine calcium antagonist — nifedipine — inhibits only the reinforcing properties of morphine but not of ethanol. Although other studies have shown that some calcium channel antagonists — nitrendipine and nimodipine, when given after ethanol withdrawal, prevented withdrawal convulsion (10), it could be suggested that ethanol-induced reward measured in the CPP paradigm, was not mediated through calcium channels. Moreover, ethanol *in vitro* was found not to affect dihydropyridine binding in brain tissue, except at very high doses (11).

On the contrary, the present data show that nifedipine influences the effect of cocaine in the CNS. Recently, it was shown that dihydropyridine calcium antagonists inhibited cocaine-induced DA release (in the mesolimbic DA system) and motor stimulation. This effect depends on their interference with calcium influx across L-type voltage-sensitive channels (9). Also cocaine-induced rewarding properties measured in the CPP paradigm depend on calcium-dependent mechanism. These results indicate that dihydropyridine calcium channel antagonists might be clinically useful for the treatment of cocaine abuse. While in human cocaine has potent rewarding effect, in rodents, after 3 days of conditioning, cocaine seems to be less potent reinforcer than morphine. In addition, in case of ethanol 10 days of conditioning are necessary.

The present data demonstrate that nifedipine and verapamil can act as weak but positive reinforcers using CPP procedure in rat. Although it has been shown that several Ca^{2+} channel blockers are neutral using CPP testing (12), the possibility exists that these drugs may affect, either by increasing or decreasing, DA release, especially in the mesolimbic system which is critical for drug-induced reinforcement. These effects require additional confirmation with the aid of other procedures.

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