Pharmacology Biochemistry & Behavior, Vol. 36, pp. 795–798. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Antidepressant-Like Action of Nicardipine, Verapamil and Hemicholinium-3 Injected Into the Anterior Hypothalamus in the Rat Forced Swim Test

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Received 14 December 1989

BIDZINSKI, A., E. JANKOWSKA AND O. PUCILOWSKI. *Antidepressant-like action of nicardipine, verapamil and hemicholinium-3 injected into the anterior hypothalamus in the rat forced swim test.* PHARMACOL BIOCHEM BEHAV **36**(4) 795–798, 1990.—Male Wistar rats, chronically implanted with cannulas into the anterior hypothalamus, were acutely injected with the calcium channel inhibitors, diltiazem, nicardipine and verapamil, or the choline uptake blocker hemicholinium-3 and tested in the forced swim test. Hemicholinium-3, nicardipine and verapamil markedly increased the duration of active swimming. This antidepressant-like effect did not appear to reflect merely a hyperactive state as the drug-treated rats did not differ from vehicle-injected controls in their open field motility scores. Diltiazem failed to influence rats' performance in either test. Since nicardipine and verapamil, but not diltiazem, share choline uptake property with hemicholinium-3, it seems that this action plays a role in the antidepressant-like effect of all three drugs in the forced swim test.

Forced swim test Open field test Diltiazem Nicardipine Verapamil Hemicholinium-3
Microinjections Hypothalamus

ANTIDEPRESSANT activity of various calcium channel inhibitors (CCI) has been demonstrated in clinical (19,32) and preclinical studies (7, 15, 25, 26). These findings have aroused understandable interest in clinicians and researchers alike. CCIs are considered to be relatively safe drugs, except for some concern about cardiodepressive action of verapamil. Conversely, antidepressant drugs currently in use produce a number of untoward effects. If the antidepressant-like activity of CCIs could be clinically established, these drugs could offer an alternative treatment possibility or perhaps reduce the doses of classical antidepressants with combined therapy. It is presumed that the antidepressant-like effect of CCIs is due to their main mechanism of action, i.e., the inhibition of calcium inward fluxes through the cellular membrane channels of the voltage sensitive L-type (22). There is no evidence so far that these channels play a role in affective disorder, although the possibility that intracellular calcium may somehow be involved has been suggested (5,12).

Calcium channel blockade may not be the mechanism of action since CCIs considerably vary in their antidepressant-like activity [cf. (7,26)]. Differences in these drugs' permeability into the CNS may partly explain the varying effectiveness of CCIs in animal tests. However, it is also known that these drugs possess pharmacological actions other than calcium channel blockade. For instance, some of them are known to inhibit synaptosomal amine uptake (24) or interact with adrenergic, muscarinic or adenosine receptors (13, 16, 27). We have recently argued that their antidepressant-like activity might be related to their potent inhibitory action on a high affinity choline uptake process (Bidzinski and Pucilowski, submitted). This property of at least some CCIs has been previously demonstrated to be a common feature of a number of classical and new generation antidepressants, e.g., amitryptiline, desipramine, chlorimipramine, mianserin or maprotyline (4). In the present study it is shown that direct intracerebral injection of the model choline uptake blocker hemicholinium-3 as

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well as two CCIs that share this activity, nicardipine and verapamil, markedly decreased immobility in a forced swim test. This test was developed to screen antidepressant drugs which reduce the immobility period of animals in a cylinder containing water (33). The model is based on the assumption that unavoidable stress precipitates depressive-like state in experimental setting, referred to as behavioral despair or learned helplessness (38). The injection site, anterior hypothalamus (AH), was chosen because it is known to play a vital role in mood and endocrine control, disorders of both being a common feature of depression (20,21). Cholinergic mechanisms seem to be involved in regulation of both. Stimulation of muscarinic receptors decreases neuronal activity in the AH (1). It also activates (14), whereas implantation of atropine crystals suppresses the release of corticotropin releasing hormone (18,23). Carbachol injected into AH facilitates shock-induced aggression in rats (3). The drug is known to induce rage reaction in cats and that effect can be completely blocked by microinjections of atropine into AH (36).

Additionally, two of the CCIs used in the present experiment, diltiazem and verapamil, have been investigated for their effects upon injection into the AH. Diltiazem caused hyperlocomotion (2) and verapamil hypothermia (35).

METHOD

Animals

Eighty-five male Wistar rats weighing 250 ± 10 g were used. The animals were maintained in individual wire mesh cages (20 × 20 × 25 cm) under 12:12 light/dark cycle (lights on at 0700 hr) with free access to commercial chow and tap water.

Surgery

Under ethyl ether anesthesia the rats were implanted with sockets containing two parallel stainless steel guide cannulas (22 gauge) their tips ending 2 mm above the injection site. The stereotaxic coordinates for the injections into the anterior hypothalamic area (AH) were: A 7.0 mm, L ±1.0 mm, V −7.5 mm from the dura (30). The sockets were fixed to the skull with metal screws and acrylic cement. A stainless steel stylet was placed in every guide cannula to prevent occlusion. Ten days were allowed for postsurgical recovery.

Injections

Injections were delivered bilaterally in hand held animals which had been handled for three days prior to the first injection session. Drug solutions or saline were microinjected in a volume of 0.5 μl per side at a rate of 1 μl/min via a 30 gauge injection cannula connected by a polyethylene tubing to a Hamilton 5 μl syringe. There were two injection sessions spaced 4 days apart. During the first one the effect of drugs upon locomotor activity in the open field was checked, during the second, behavior in the forced swim test. It is known that exposing the animals to the open field immediately prior to elevated plus maze test of anxiety results in increased activity in the latter test (31). However, based on the results of pilot experiments, four days between test interval in our study ought to be sufficiently long as to prevent any influence of open field testing upon performance in the forced swim model.

Procedures

Open field test. Immediately after microinjection each rat was placed in the center of a circular arena 100 cm in diameter of an automatic open field device (COTM, Bialystok, Poland). A single 60-W white bulb, hung 2 m above the center of the arena, provided the light source. The rat was allowed 15 min to habituate to the apparatus. Locomotor activity (the number of photobeam crossings) was then automatically recorded over the next 15-min period.

Forced swim test. Three days after the open field test each rat was placed in a glass tank (15 cm diameter, 60 cm high) filled up to 20 cm with warm (25°C) water and left to habituate for 5 min. Twenty-four hr later the rats were microinjected and after a 20-min interval the duration of swimming activity was recorded with a cumulative stop watch over a 5-min period by direct observation. A rat was considered to stop swimming when at least three paws were immobile. The observer was unaware of the type of treatment applied to each rat.

Rats were randomly assigned to the saline- or drug-treated groups. After completing the experiments, the animals were sacrificed by an overdose of chloroform, their brains removed and the cannula location verified histologically.

Drugs

The following compounds were used: hemicholinium-3 (Serva, Heidelberg, FRG) in a dose of 0.1 and 0.5 nmoles per side; diltiazem HCl (Goedecke, Freiburg, FRG) 0.1 or 0.5 nmoles/side; nicardipine HCl (Yamanouchi, Tokyo, Japan) 0.025 pmoles, 0.025 nmoles or 0.1 nmoles/side; and verapamil HCl (Lek, Ljubljana, Yugoslavia) 0.1 or 0.5 nmoles/side. All drugs were dissolved in redistilled water. Nicardipine handling was done under protective sodium light.

Statistics

The data were analyzed using a one-way ANOVA followed by the Dunnett's test. All data are presented as means ± standard error of the mean.

RESULTS

All implanted rats had their guide cannulas within the upper limit of the AH and consequently could have been included into data analysis. However, some animals had to be rejected because of supradural abscesses.

Open Field Test

There was no significant between group difference in the rat's motility as revealed by ANOVA, $F(9,68) = 2.03$.

Forced Swim Test

The ANOVA showed a significant overall change in swimming activity following microinjections into the AH, $F(9,68) = 3.93$, $p<0.01$. Hemicholinium-3, verapamil and two higher doses of nicardipine significantly prolonged the time spent swimming as compared to saline-injected controls. Diltiazem failed to influence rats' behavior in the test.

DISCUSSION

We have found that acute hemicholinium-3 microinjection significantly prolonged active swimming (antidepressive-like effect) without changing rats' activity in the open field. This drug acts by inhibiting choline uptake at both muscarinic and nicotinic cholinergic junctions thereby decreasing the synthesis of acetylcholine and in consequence its release at the synapse. Our results are in agreement with the evidence that supersensitivity to cholinergic stimulation, brought about by either selective breeding (28) or antimuscarinic treatment (29), is accompanied by increased

TABLE 1

EFFECT OF INTRAHYPOTHALAMIC INJECTIONS OF DILTIAZEM, NICARDIPINE, VERAPAMIL AND HEMICHOLINIUM-3 ON RATS' BEHAVIOR IN THE OPEN FIELD AND FORCED SWIM TEST (MEANS ± SE)

Treatment	Drug Dose (per side)	n	Open Field Activity Count	Forced Swim Time (sec)
Saline	0	15	31 ± 6	20 ± 4
Hemicholinium	0.1 nmoles	7	42 ± 9	46 ± 7*
	0.5 nmoles	8	23 ± 6	62 ± 4*
Nicardipine	0.025 pmoles	6	66 ± 18*	19 ± 4
	0.025 nmoles	6	58 ± 23*	52 ± 23*
	0.1 nmoles	7	54 ± 5*	49 ± 7*
Verapamil	0.1 nmoles	6	32 ± 7	61 ± 23*
	0.5 nmoles	7	41 ± 8	79 ± 22*
Diltiazem	0.1 nmoles	8	35 ± 8	25 ± 5
	0.5 nmoles	7	23 ± 7	26 ± 3

n = number of animals; *$p<0.05$, vs. Saline, Dunnett's test.

immobility in the forced swim test. The present results are consistent with earlier reports showing that CCIs have antidepressant-like properties in some animal tests (7, 15, 25, 26). We have now added the evidence that the AH, previously demonstrated to contain high concentrations of CCI binding sites (11), forms at least one neural site for this behavioral activity.

The behavioral evidence of antidepressant-like action of CCIs brought some controversy. If calcium channel blocking activity of these drugs could account for that effect then all CCIs should be similarly active in animal tests. The existing evidence points to something different [cf. (7, 25, 26)]. Although dihydropyridine derivatives like nifedipine, nimodipine or nicardipine seem to share antidepressant-like properties, diltiazem, a benzothiazepine CCI, failed to show such activity in the forced swim test (26). Similarly, varying effectiveness of different CCIs, even within the dihydropyridine class, has been reported for a number of behavioral tests other than depression models (8, 9, 17, 34). One may conclude that the antidepressant and possible other behavioral effects of CCIs do not necessarily reflect their calcium channel blocking potency.

An alternative hypothesis of the mechanism of antidepressant drug action concerns their anticholinergic activity (10). There are, of course, some differences between antidepressant drugs in their antimuscarinic action (6). Based upon our previous evidence (4) we assume that anticholinergic activity of antidepressant drugs may depend upon choline uptake inhibition as well as on muscarinic receptor blocking effect. We have recently investigated the choline uptake blocking effect of three putative 'antidepressants' from different CCI classes (Bidzinski and Pucilowski, submitted). We found that nicardipine and verapamil inhibit choline uptake in human erythrocytes with potency comparable to that of hemicholinium-3. Diltiazem was only marginally effective after a 20-min preincubation time. Interestingly enough, in the present study, acutely administered diltiazem failed to influence open field or forced swim behavior, whereas verapamil and nicardipine significantly prolonged swimming time. Hemicholinium, although obviously not regarded as an antidepressant, nevertheless has been presently found to produce an antidepressant-like behavioral effect in the forced swim test. These data suggest that it is possible that the acute inhibition of choline uptake produces antidepressant associated effects since hemicholinium-3, nicardipine and verapamil all inhibit choline uptake (but not calcium channel function). The present data support the earlier evidence favoring a cholinergic theory of depression and antidepressant drug activity (10,20). However, certainty awaits the completion of dose-effect studies.

Although ANOVA did not reveal significant changes in the overall influence of the tested drugs on activity in the open field it should be noted that at least nicardipine appeared to increase motility in the test (all doses significantly enhanced activity according to Dunnett's test). This effect may be attributed to anxiolytic-like action of dihydropyridine CCIs in low doses (8). Peripherally administered higher doses of CCIs induce clear sedation (17). It seems to be the case also with microinjections of nicardipine into the AH, as the activity score decreased with dose. However, if increased general activity was to account for nimodipine's antidepressant-like action in the forced swim test, the drug should be most effective in the lowest dose, just as in the open field. It seems possible instead that anxiolytic action could be conducive to presently observed antiimmobility effect in the forced swim test, as its potential anxiogenic component was already suggested (37). Considerable variability of individual responses to the 0.025 nmole dose of nicardipine was responsible for lack of clear dose-response effect in the forced swim test.

One should note that the doses of compounds used in the present study were deliberately chosen to be considerably lower than commonly employed in similar studies, e.g., 125 μg of diltiazem in the study by deBeaurepaire and Freed (2). When the drugs produce behavioral effect in doses as low as 0.05 or 0.25 μg per injection site (the highest doses of nicardipine and verapamil, respectively), it is very likely that it is a specific effect. This study more directly supports the hypothesis that the antidepressant-like effects of CCIs is central one and related to these drugs' action on hypothalamic neurons.

ACKNOWLEDGEMENTS

The study was partially supported by the Polish Ministry of Health grant RPBR MZ.V-R.5.3. Gifts of diltiazem from Goedecke (Freiburg), nicardipine from Yamanouchi (Tokyo) and verapamil from Lek (Ljubljana) are highly appreciated. We thank Prof. Burr Eichelman and two editorial referees for criticisms and comments made on the earlier version of this paper.

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