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SHORT COMMUNICATION

Effects of Calcium Antagonists Nifedipine and Flunarizine on Phencyclidine-Induced Changes in the Regional Dopaminergic Metabolism of the Rat Brain

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ABSTRACT. To elucidate the psychotropic actions of calcium (Ca) antagonists, we investigated the effect of the voltage-dependent Ca channel antagonists, nifedipine and flunarizine, on phencyclidine (PCP)-induced changes in the monoamine metabolism in the regional brain areas of rats. The results indicate that the administration of nifedipine alone attenuated dopamine (DA) metabolism in the nucleus caudatus putamen while enhancing serotonin (5-HT) metabolism. By contrast, flunarizine increased DA metabolism. PCP significantly increased DA metabolite levels in the prefrontal cortex, the nucleus caudatus putamen, and the amygdala. The PCP-induced increases in DA metabolism in these regions were significantly antagonized by nifedipine, but not by flunarizine. These results indicate that nifedipine attenuates the PCP-induced hyperactivity of the dopaminergic neurons, suggesting antipsychotic properties for this drug. BIOCHEM PHARMACOL 51;1:83–86, 1996.

KEY WORDS. calcium antagonist; phencyclidine; dopaminergic neuron; nifedipine; flunarizine

Recently, Ca§ antagonists have been utilized in the treatment of various neuropsychiatric disorders, such as affective disorders, schizophrenia, and tardive dyskinesia. Studies have suggested the possibility that Ca antagonists exert a direct influence on the CNS, *via* an alteration of the central blood flow, when effecting their psychotropic action [1]. Previous studies have indicated that Ca antagonists could act, not only on amino acid neurotransmitter systems, but also on monoaminergic systems such as the DA system and 5-HT systems in the CNS [1, 2]. The Ca antagonists nifedipine and flunarizine have been shown to block amphetamine-induced behavioral stimulation in mice [3]. Neurochemically, such compounds have been demonstrated to decrease both DA synthesis in the mouse brain [4] and DA metabolite levels in the rat brain [5]. These reports have also suggested that Ca antagonists have the capability of interfering with dopaminergic transmission.

PCP is one of the major drugs involved in substance abuse in the U.S.A. This drug has received attention because it can induce a psychotic state that closely resembles schizophrenia [6]. PCP has been suggested to be useful as a drug model of psychosis. Actually, PCP induces both hyperlocomotive activity and stereotypic behavior in rodents and also enhances DA metabolism in specific regions of the rodent brain [7]. In addition, both pharmacological and neurochemical studies have suggested that PCP interacts to some extent with Ca antagonists [8]. Ca antagonists belonging to different chemical classes have been found to modulate these PCP-induced behavioral changes in experimental animals [9, 10].

Nevertheless, the effects of Ca antagonists on the PCP-induced neurochemical changes in the DA systems still remain unclear. To elucidate the psychotropic effects of Ca antagonists, we investigated the effects of the Ca channel antagonists nifedipine and flunarizine on regional DA metabolism in the rat brain following systemically administered PCP. Nifedipine is one of the dihydropyridines mainly binding to the L-type Ca channel and flunarizine has been reported to have the capability of blocking either the T- or N-type Ca channel [1, 11, 12]. It was hypothesized that both drugs have distinct effects on PCP-induced changes in DA metabolism *via* action on different types of Ca channel.

MATERIALS AND METHODS

Eight-week-old male Wistar Kyoto rats weighing 240–300 g were used. Nifedipine and flunarizine hydrochloride were obtained from the Funakoshi Chemical Co. (Tokyo, Japan) and were dissolved in a vehicle (10% ethanol and 10% Tween 80 in saline, respectively). Phencyclidine hydrochloride, the generous gift of Prof. Nabeshima, Nagoya University, was

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§ Abbreviations: Ca, calcium, CNS, central nervous system; DA, dopamine; PCP, phencyclidine; 5-HT, serotonin; HPLC-ECD, high-performance liquid chromatography with electrochemical detection; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid.

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dissolved in saline. The choice of dose and pretreatment time of Ca antagonists was based on the results of previous studies [5, 13].

The rats were pretreated with either nifedipine 20 mg/kg, intraperitoneally (i.p.), flunarizine 20 mg/kg i.p., or the vehicle 60 min prior to the administration of PCP 5.0 mg/kg i.p. At the same time, saline was administered to a part of the group of rats pretreated with Ca antagonists to verify the effects of the Ca antagonists alone. A control group of rats was pretreated with the vehicle 60 min prior to saline. All of the rats were sacrificed by decapitation 60 min after the administration of either PCP or saline. The brain was quickly removed and frozen and stored at −80°C. The regional brain tissue was carefully dissected by hand with a microknife. DA, 5-HT, and their metabolites were simultaneously determined by using HPLC-ECD as previously described [14]. All data were subjected to a one-way analysis of variance followed by Student's *t* test. The difference between the means were considered significant at $P(F) < 0.05$ by analysis of variance combined with $P < 0.05$ for Student's *t*-test.

RESULTS

Figure 1 shows the effects of nifedipine and flunarizine on DOPAC and HVA levels in the discrete regions of the rat brain 60 min after PCP 5.0 mg/kg i.p. In the saline-pretreated rats, PCP 5.0 mg/kg significantly increased DOPAC (+82%) and HVA (+34%) in the prefrontal cortex; DOPAC (+43%) in the nucleus caudatus putamen; and DOPAC (+46%) and HVA (+46%) in the amygdala. Nifedipine alone significantly reduced both DOPAC (−15%) and HVA (−34%) levels only in the nucleus caudatus putamen, compared to the control group. Pretreatment with nifedipine significantly lowered the PCP-induced increases in DOPAC in the nucleus caudatus putamen, in DOPAC and HVA in the prefrontal cortex, and in HVA in the amygdala to the control levels or less. A similar trend of the attenuation by nifedipine of the effect of PCP was also found in DOPAC in the anterior cingulate cortex.

Flunarizine alone significantly increased both DOPAC (+46%) and HVA (+42%) levels in the nucleus caudatus putamen and DOPAC (+35%) in the prefrontal cortex, compared to the control group. These effects were still observed in the group of rats receiving both flunarizine and PCP. Moreover, pretreatment with flunarizine significantly enhanced the PCP-induced increases in HVA in the prefrontal cortex and in DOPAC and HVA in the amygdala.

PCP, Ca antagonists, and the combination of both drugs had no significant effect on DA levels in any brain regions examined.

Nifedipine, but not flunarizine, significantly increased 5-HT levels in the amygdala (+21%) and 5-HIAA levels in the nucleus caudatus putamen (+25%), the nucleus accumbens (+28%), and the hippocampus (+23%) compared to the control group of rats. PCP had no significant effect on either 5-HT or 5-HIAA levels in any brain regions. No interaction between PCP and Ca antagonists with regard to 5-HT systems was found.

DISCUSSION

It was formerly believed that the effect of Ca antagonists on the CNS is mainly produced as a secondary result following changes in the blood circulation because of its difficulty in penetrating the blood-brain barrier, but these assumptions are now being reevaluated [1]. For administration of Ca antagonists alone, we observed that nifedipine attenuated DA metabolism in the nucleus caudatus putamen while enhancing 5-HT metabolism. By contrast, flunarizine increased DA metabolism in the prefrontal cortex and the nucleus caudatus putamen. These results are consistent with previous findings [5, 13]. Therefore, the present results should be regarded as demonstrating the direct effect of Ca antagonists on the monoaminergic neurons.

In this study, the PCP-induced increases in DA metabolism in the prefrontal cortex, the nucleus caudatus putamen, and the amygdala were significantly antagonized by pretreatment with nifedipine 60 min prior to PCP. These results indicated that nifedipine attenuated the PCP-induced hyperactivity of the dopaminergic neurons in specific regions of the rat brain. Several studies have previously reported the antagonistic effect of Ca antagonists on the neurochemical changes in dopaminergic systems induced by psychotropic agents other than PCP [13, 15]. Pani *et al.* [15] indicated that dihydropyridine Ca antagonists may attenuate the enhancement of Ca-dependent DA release. Accordingly, it may be speculated that PCP, like cocaine, enhances DA release by a Ca-dependent mechanism. However, it is unlikely that nifedipine inhibits the PCP-induced DA release by its interference with Ca influx into the nerve terminals *via* L type Ca channels, for which nifedipine has high affinity and specificity. Recent studies on the distribution of Ca channel subtypes have shown that L-type Ca channels are located only in neuronal cell bodies or at the base of dendrites [11]. Otherwise, dopamine release from mesencephalic neuronal cell cultures was reported to relate to the Ca^{2+} dependent mechanism regulated by the N-type channel, but not the L-type channels [16]. Therefore, nifedipine may mainly bind to the L-type channel on cell bodies of the dopaminergic neurons to suppress the neuronal firing. Further investigations on the physiological role of the L-type channel on cell bodies are needed to elucidate this mechanism.

Flunarizine did not attenuate, but, instead, enhanced the increase in DA metabolism induced by PCP in the specific brain regions; such enhancement could be a result of its antidopaminergic effects. Previous reports have suggested that this Ca antagonist inhibited presynaptic DA receptors, probably due to the structural similarity of the flunarizine molecule with that of classic neuroleptics [13], and had a neuroleptic-like action on DA release [15]. Therefore, pretreatment with flunarizine might bring about an additional increase in the augmented DA metabolism following PCP by the blockage of pre-

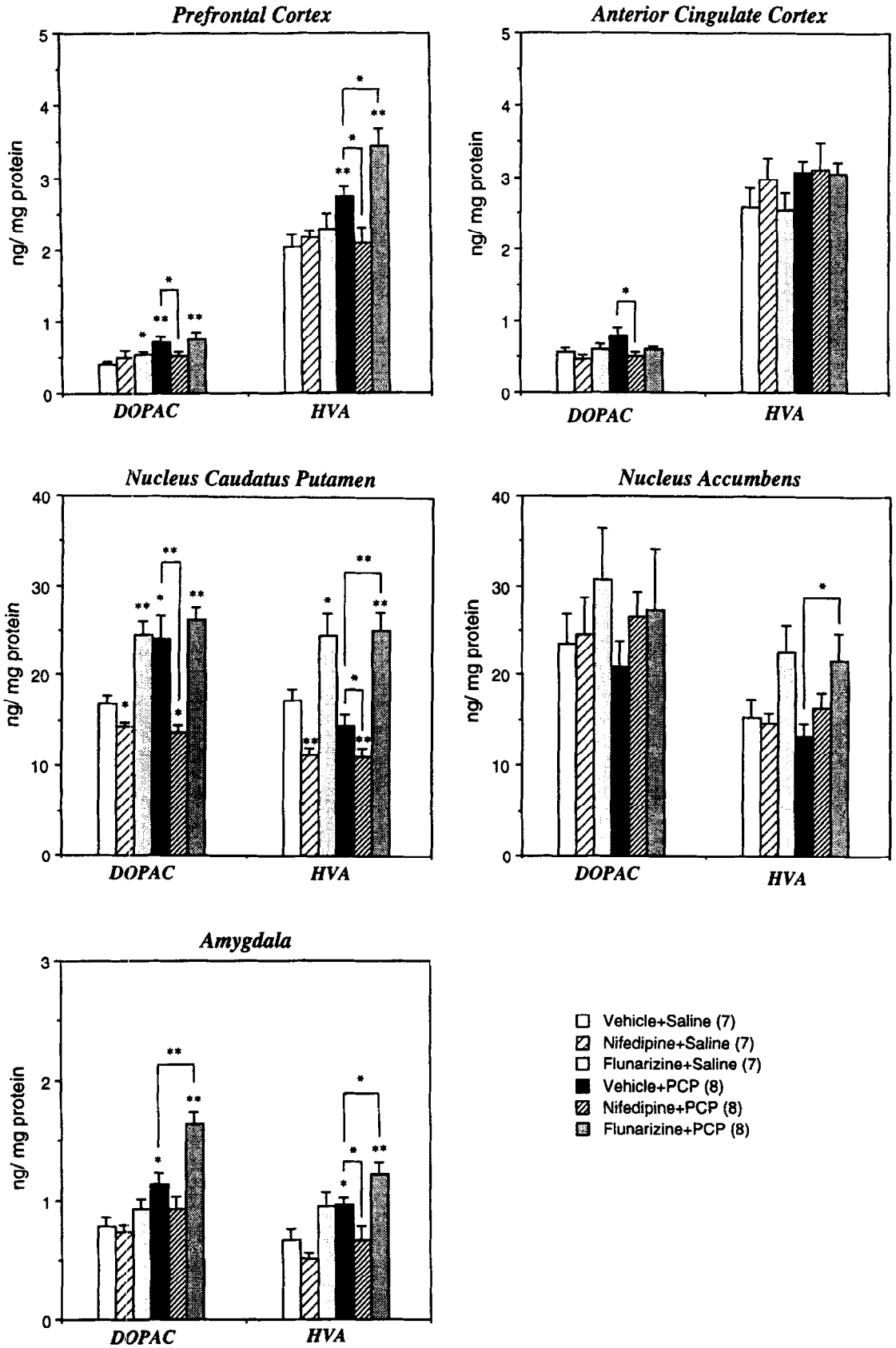

FIG. 1. The effect of calcium antagonists on the 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels in the discrete regions of the rat brain following PCP 5.0 mg/kg i.p. The data are expressed as the mean ± SEM (ng/mg protein). The number of determinations are noted in parentheses.

*$P < 0.05$, **$P < 0.01$ compared to the control group of rats receiving the vehicle and saline by Student's *t*-test following a one-way analysis of variance.

and postsynaptic DA receptors with flunarizine. Moreover, according to electrophysiological studies, flunarizine appears to inhibit either T-type or N-type Ca channels [12]. The difference in the specificity for Ca channels may also contribute to the assumption that flunarizine has a different effect on the dopaminergic neurons than does nifedipine.

In conclusion, the present results demonstrated that nifedipine, but not flunarizine, significantly antagonized the PCP-induced increases in DA metabolism in specific brain regions. These findings indicate that nifedipine attenuates the PCP-induced hyperactivity of specific DA systems, suggesting antipsychotic properties for this drug. This interference with DA systems contributes to the clinical efficacy of Ca antagonists in the treatment of both psychiatric and neurological disorders.

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