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The Iminodipropionitrile (IDPN)-Induced Dyskinetic Syndrome in Mice: Antagonism by the Calcium Channel Antagonist Nifedipine

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CADET, J. L., E. TAYLOR AND W. J. FREED. *The iminodipropionitrile (IDPN)-induced dyskinetic syndrome in mice: Antagonism by the calcium channel antagonist nifedipine.* PHARMACOL BIOCHEM BEHAV **29**(2) 381–385, 1988.—Chronic administration of IDPN leads to the development of a persistent syndrome which is characterized by lateral and vertical neck dyskinesias, random circling behaviors, and locomotor hyperactivity. Although the dihydropryridine (DHP) calcium channel antagonist nifedipine inhibited all aspects of the syndrome, lateral head dyskinesias (laterocollis) and circling abnormalities were the most significantly affected signs. Dysregulation of calcium-dependent processes might be involved in the pathogenesis of the IDPN-induced dyskinetic abnormalities and clinical disorders of movement in humans.

Iminodipropionitrile Serotonin syndrome Phencyclidine Nifedipine BAY K 8644
Calcium channels Dihydropyridine binding site Dystonias Spasmodic torticollis

IMINODIPROPIONITRILE (IDPN) is a neurotoxin which causes permanent neuropathological changes in both the central (CNS) and the peripheral nervous system [5–9, 11, 19, 37]. Animals treated with this drug may develop disturbances in their righting reflexes and a gait disorder which consists of dragging of the hindlimbs. These abnormalities are thought to be related to the toxic effects of IDPN on slow transport of neurofilament proteins [19]. More recently, it was reported that the compound may also affect retrograde axonal transport of proteins that might be necessary for the proper functioning of neurons [11].

Chronic treatment with IDPN also leads to the development of a complex motor syndrome which consists of hyperactivity, increased startle response, lateral and vertical head spasmodic dyskinesias, and random circling [5–9, 11, 14, 37]. These abnormalities become apparent by the third to the seventh day of IDPN administration and last throughout the lifetime of the animals without significantly affecting their longevity. This behavioral syndrome has been termed the "ECC-syndrome" (excitation, chorea, circling) by Selye [37] and, more picturesquely, the "waltzing syndrome" by Chou and Hartmann [9]. The IDPN-induced changes are very similar to those observed after acute administration of serotonin agonists [2, 18, 20, 22] and phencyclidine [12,18] (see Table 1). Moreover, the abnormalities caused by these agents appear also to have very comparable neuropharmacological profiles [2, 6, 7, 12, 14, 18]. Because of its persistence and its resemblance to the phenomena seen in some human disorders of the basal ganglia, some authors have proposed that the waltzing syndrome be used as a possible model of clinical movement disorders [6, 14, 37].

Both the 5-HT- and the PCP-induced abnormalities have been used as model to study neurotransmitter interactions in the CNS. In humans, PCP can cause behavioral changes which consist of psychotic and motor abnormalities [33]. These may include a schizophrenia-like psychosis, violent outbursts, and stereotypic behaviors [33]. In animals, PCP causes increased locomotor activity, stereotypic behaviors, and ataxia [12,28]. It has been proposed that an understanding of the basic mechanism involved in the actions of PCP may help to shed some light on the human disorders that the drug mimics [33]. Although the existence of high affinity

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TABLE 1
COMPARISON OF THE BEHAVIORAL PHENOMENA RESULTING FROM IDPN, PCP, AND 5-HT AGONISTS

	IDPN	PCP	5-HT Agonists
Hyperactivity	+	+	+
Ataxia	+	+	+
Lateral head weavings or twitches (laterocollis)	+	+	+
Backward head tilting (retrocollis)	+	−	±
Random circling	+	±	±
Walking backwards	±	±	+
Forepaw treading	±	±	+
Stereoptypies	+	+	+
Increased startle response	+	±	±
Somersaulting	±	−	−
Persistence	+	−	−

+=present; ±=may be present; −=absent.
Modified from [6].

binding sites for PCP has been demonstrated in the CNS, not all of its effects can be directly linked to its interactions with these receptors [1]. For instance, several neurotransmitters including dopamine [12,28], serotonin [29], and some peptides [21,39] have been implicated in the manifestations of the PCP-induced behavioral changes. Although a similar situation may exist for the IDPN- [6–8, 14] and the 5-HT-induced [2, 18, 20] abnormalities, it is worth noting that no changes in PCP binding sites were found in the brain of IDPN-treated rats [35]. We do not know of any study where the characteristics of the PCP binding sites were evaluated after chronic treatment with 5-HT agonists.

Recently, it was reported that PCP can interact with binding sites of the dihydropyridine (DHP) calcium antagonists and lead to an increase in the affinity of the receptor in rat brain [3]. Other studies have also shown that DHP antagonists such as nifedipine can block PCP-induced locomotor activation in mice [16]. Because the PCP-induced behavioral activation is often used as a model of neuropsychiatric disorders, it was suggested that nifedipine might have a role to play in the treatment of human neuropsychiatric and movement disorders [16]. Both DHP [26] and PCP [34] binding sites are indeed found in areas of the brain such as the caudate and the frontal cortex which are linked to motor and psychotomimetic phenomena. Interestingly, it has been reported that the DHP calcium channel agonist BAY K 8644 can cause a behavioral syndrome which consists of ataxia, straub tail, increased tonus in forelimbs and hindlimbs, backward locomotion, and increased sensitivity to auditory stimulation in both mice [4] and rats [32]. These behavioral abnormalities represent some of the same changes seen after treatment with IDPN, PCP, or 5-HT agonists (see Table 1). The BAY K 8644-induced behaviors were inhibited by nifedipine but not by other calcium antagonists [4,32].

In order to continue our studies on the behavioral pharmacology of the IDPN-induced syndrome and to evaluate the idea that the drug may be beneficial in the treatment of human hyperkinetic syndromes, nifedipine was tested for its ability to antagonize various aspects of the waltzing syndrome.

METHOD

Female Swiss-Webster mice weighing between 20–30 g were used. They were kept on a 12-hr light-dark cycle and were given water and food ad lib. At the beginning of the experiment, all animals received daily intraperitoneal (IP) injections of IDPN (100 mg/kg). All the animals developed the dyskinetic abnormalities by the fifth day of injection and received no more IDPN throughout the duration of the study.

After an interval of one month, dyskinetic abnormalities were tested in the following manner. The number of lateral and vertical dyskinetic neck movements were counted twice for one minute during a 30 minute period. Circling behavior was rated on a severity scale from 0 to 3: 0=normal behavior or no circling; 1=mild; 2=moderate; 3=severe. Locomotor activity was monitored for 30 minute intervals using a photo-cell apparatus as described previously [12]. Both horizontal and vertical activities were monitored. The animals were tested by an observer who was blind to the treatments used.

On the day of observation, each mouse was put in an individual cage. The animals were left undisturbed for 30 minutes before testing began. After a 30 minute baseline, the monitoring was stopped for 5 minutes while the vehicle or one of the 3 doses of nifedipine (3.125, 6.25, 12.5 mg/kg) were injected IP. The animals were then observed for another 30 minutes. All the animals received the vehicle and all the dosages of nifedipine in a counterbalanced fashion. There was at least a four-day period between testing. Nifedipine was dissolved in 10% Tween 80 in saline in a volume of 10 ml/kg.

The data were analyzed by a repeated analysis of variance. Post-hoc analyses were done using Student's t-test since all the animals got vehicle infusion. The null hypothesis was rejected at $p<0.05$.

RESULTS

Effects of Nifedipine on IDPN-Induced Circling

Nifedipine caused significant inhibition of circling behavior (Fig. 1A). There were significant inhibitory dose effects, $F(3,28)=5.14$, $p<0.0059$, which increased with the passage of time, $F(1,28)=10.69$, $p<0.0029$. Vehicle alone caused no changes in circling behaviors seen in IDPN-treated animals whereas the highest dose of nifedipine (12.5 mg/kg) led to a reversal of circling towards normal baseline. The time by dose intereaction also increased with larger doses of the drug ($p<0.04$).

Effects of Nifedipine on IDPN-Induced Lateral and Vertical Head Movements

Nifedipine significantly antagonized both the lateral (laterocollis) and the vertical (retrocollis) head movements (Fig. 1B). There were significant inhibitory dose effects, $F(3,28)=3.56$, $p<0.0268$, on lateral head twitches. The antagonistic effects increased with time, $F(1,28)=18.86$, $p<0.0002$. Furthermore, the increase in the effects of nifedipine with time was significantly potentiated by larger doses of the drug ($p<0.00034$). The inhibitory effects of nifedipine on vertical neck dyskinesias were not dose-

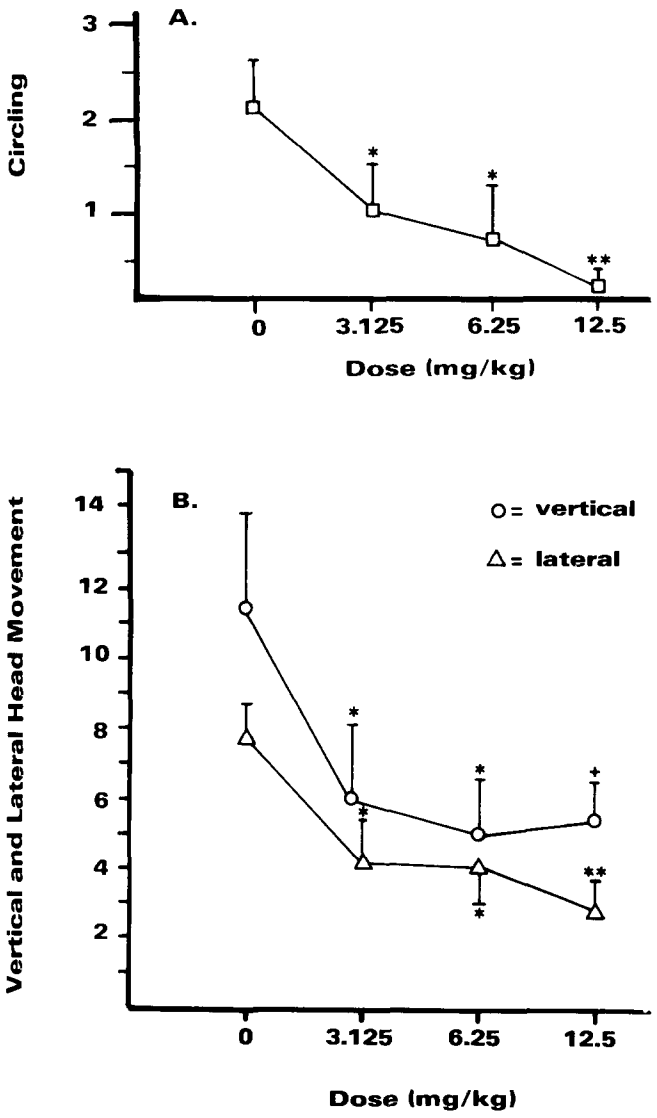

FIG. 1. Influence of various doses of nifedipine on (A) circling and (B) lateral and vertical neck movements. The values represent means±SEM obtained during the 30 min period of observation after nifedipine administration. There were 8 animals in each group. Key: +=$p<0.08$; *=$p<0.05$; **=$p<0.005$ as compared to vehicle alone (Student's t-test).

dependent with the doses employed in this study ($p<0.10$) but they increased with time, $F(1,28)=6.44$, $p<0.017$. There was no significant time × dose interaction ($p=0.27$).

Effects of Nifedipine on IDPN-Induced Hyperactivity

Both horizontal and vertical locomotor activity were also suppressed by nifedipine (Table 2). The inhibitory effects of dose were significant for horizontal activity, $F(3,28)=4.62$, $p<0.0095$. The inhibitory effects of nifedipine increased with time, $F(1,28)=22.08$, $p<0.0001$. There was also a potentiation of the effects of time with increasing doses of the drug ($p<0.0081$). Although the inhibitory effects on vertical locomotor activity were not dose-dependent, $F(3,28)=1.87$, $p<0.16$, they increased with time, $F(1,28)=5.87$, $p<0.02$.

TABLE 2

EFFECTS OF NIFEDIPINE ON LOCOMOTOR ACTIVITY IN IDPN-TREATED MICE

	Vertical Movements		Horizontal Movements	
Dose (mg/kg)	Baseline	After Dose Indicated	Baseline	After Dose Indicated
0.0	510 ± 291	844 ± 304	919 ± 279	1083 ± 228
3.125	694 ± 288	292 ± 130	1291 ± 214	447 ± 152*
6.250	941 ± 471	440 ± 331	1030 ± 241	453 ± 179*
12.500	870 ± 458	73 ± 38†	1355 ± 297	226 ± 104†

The values represent means ± SEM of 30 min of activity counts pre- and post-nifedipine. There were 8 animals in each group.
*$p<0.05$; †$p<0.005$ in comparison to saline (Student's t-test).

There a slightly significant inhibitory time × dose intereaction with increasing doses of nifedipine ($p<0.058$).

DISCUSSION

The DHP calcium channel antagonist nifedipine blocks to varying degrees all aspects of the IDPN-induced abnormalities tested in the present study. The inhibitory effects were apparent within a few minutes after the administration of the drug. The antagonistic actions of the drug on lateral head movements (laterocollis) were greater than those on the vertical ones (retrocollis). Horizontal locomotor activity was also more affected than vertical activity as measured by the photocell apparatus. The most complete effect of the drug was on circling which was almost totally abolished at the higher dose (12.5 mg/kg).

Possible Role of Calcium-Dependent Processes in the Manifestations of the IDPN-Induced Syndrome

The present findings on the effects of nifedipine on IDPN-induced hyperactivity extend the previous reports that the DHP calcium channel antagonist nifedipine can block both PCP-[16] and amphetamine-induced [17] hyperactivity in mice. Our data on IDPN-induced circling are also consistent with a previous report that calcium channel inhibitors can inhibit circling behavior elicited by amphetamine in mice with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathways [13]. Furthermore, they are consistent with the reports that the calcium channel activator BAY K 8644 can cause a set of nifedipine-responsive behavioral abnormalities which are reminiscent of the IDPN-, PCP-, and 5-HT-induced syndromes in mice [4] and rats [32]. Although it is not presently clear how nifedipine attenuated the manifestations of the IDPN-induced syndrome in the present experiment, its locus of action is likely to be at the level of the DHP binding sites [15,31] which are localized in several areas of the brain such as the frontal cortex and the caudate-putamen which are related to motor phenomena [26]. Involvement of other brainstem structures such as the raphe nuclei needs to be considered in view of the phenomenology of the IDPN-induced abnormalities.

The present data as well as other reports [16, 17, 38] indicate that calcium-dependent processes may be involved in the manifestation of PCP-, amphetamine-, and IDPN-induced hyperkinetic abnormalities, possibly via similar

mechanisms. This idea is consistent with our previous demonstration that the brain of IDPN-treated rats show a significant increase in the maximal number of alpha-1 adrenergic receptors in their caudate-putamen, a change which was associated with a concomitant increase in norepinephrine (NE)-stimulated phosphotidyl inositol turnover in the striatum [5]. This second messenger system plays a significant role in the regulation of calcium-dependent processes via protein kinase C [30]. Taken together, these data indicate that IDPN may be causing alterations in calcium channels by increasing the phosphorylation state of the receptor protein through the activation of the phospholipid second messenger system [5]. Activation of these channels may lead to the IDPN-induced syndrome in a fashion similar to the dyskinetic behaviors observed after injection of the calcium activator BAY K 8644 [4,32]. Thus, nifedipine may antagonize the effects of IDPN in two ways. First, nifedipine, by blocking the activated calcium channels, may lead to a decrease in calcium influx into cells responsible for the manifestations of the syndrome, in a manner similar to its inhibition of the BAY K 8644-induced abnormalities [4,32]. Secondly, by reducing calcium influx into noradrenergic or serotonergic cells, nifedipine can inhibit the release of the monoamines thus indirectly antagonizing, for example, the stimulation of the up-regulated alpha-1 adrenergic receptors observed in the brains of IDPN-treated animals [5]. This hypothesis is consistent with our previous demonstration that the alpha-1 adrenergic antagonist prazosin can also block the manifestations of the IDPN-induced syndrome [6].

Possible Clinical Implications

In addition to their similarities to the PCP- and 5-HT-induced syndromes, the IDPN-induced phenomena also mimic some of the dyskinetic signs observed in some human movement disorders such as the dystonias [6,10]. The dystonias can be either primary (idiopathic) or secondary to a number of causes including drug treatment with antipsychotics [23] or a number of metabolic abnormalities including organic acidurias [24]. Although more work needs to be done on the biochemistry of the IDPN model before it can be proposed as a model of human movement disorders, the finding that nifedipine causes significant inhibition of persistent lateral and vertical head twitches raises the possibility that this drug might be beneficial in the treatment of primary and/or secondary dystonias which are characterized by similar movements. Since the present approach to the treatment of hyperkinetic syndromes is primarily symptomatic and usually troublesome [10, 23, 27], the use of nifedipine which is relatively well tolerated even by patients with cardiac diseases [25] may offer an alternative in the care of these patients.

In conclusion, the present results add further support for possible clinical trials of the DHP calcium channel antagonist nifedipine in the treatment of human hyperkinetic disorders. This conclusion is consistent with the demonstration of DHP binding sites in areas of the brain that are linked to motor phenomena and with the fact that calcium seems to play a regulatory role in various aspects of neurotransmission including stimulus-secretion coupling [36] and protein phosphorylation [30]. Finally, the present results identify yet another system that might be perturbed in animals treated with IDPN. Biochemical studies on the characteristics of the DHP binding sites in IDPN-treated animals may help to identify the nature of these changes.

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