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A comparison between the non-competitive NMDA antagonist dizocilpine (MK-801) and the competitive NMDA antagonist D-CPPene with regard to dopamine turnover and locomotor-stimulatory properties in mice

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Summary. Following intraperitoneal administration of the non-competitive N-methyl-D-aspartate (NMDA) antagonist dizocilpine (MK-801), levels of the dopamine (DA) metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) increased in mouse striatum and limbic forebrain. When dizocilpine was given to animals treated with NSD 1015, an inhibitor of 3,4-dihydroxyphenylalanine (DOPA) decarboxylase and monoamine oxidase, there was an increase in levels of DOPA and 3-methoxytyramine (3-MT). These findings suggest that dizocilpine stimulates DA synthesis and release in mouse brain. Following dizocilpine treatment a clear-cut increase in spontaneous locomotor activity was observed, probably partly due to enhanced dopaminergic tone. The competitive NMDA antagonist D-CPPene produced locomotor stimulation as well, but in contrast to following dizocilpine treatment levels of 3-MT decreased. Thus the stimulation of locomotor activity following D-CPPene treatment does not seem to be mediated through activation of central dopaminergic systems. However, haloperidol pretreatment antagonized this locomotor response, indicating that the dopaminergic system plays a permissive role in this context.

Keywords: Dizocilpine, D-CPPene, NMDA receptors, dopamine metabolites, locomotion, mouse.

Introduction

Previous work has shown that N-methyl-D-aspartate (NMDA) antagonists induce locomotor stimulation in rodents and that this effect partly depends on central catecholaminergic transmission. Clineschmidt et al. (1982 a) have shown that locomotor activity in mice is increased by the non-competitive NMDA

antagonist dizocilpine (MK-801), and that the dopaminergic antagonist haloperidol and the α_1-adrenergic antagonist prazosin block this effect. The competitive NMDA antagonist AP-5 (DL-2-amino-5-phosphonovaleric acid), injected into rat antero-dorsal striatum, induces sniffing and locomotion, which are reduced by haloperidol and the atypical neuroleptic clozapine (Schmidt, 1986). Locomotor stimulatory properties of the competitive NMDA antagonist CGS-19755 (cis-4-phosphonomethyl-2-piperidine-carboxylic acid), a conformationally restricted AP-5 analogue, have also been reported in both rats and mice (Bennet et al., 1989).

However, it has also been shown that the non-competitive NMDA antagonists dizocilpine and phencyclidine (PCP), as well as the competitive NMDA antagonist D-CPPene (3-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid), can produce locomotor activity in akinetic, *monoamine-depleted* mice, i.e. mice pretreated with the monoamine depleter reserpine and the catecholamine synthesis inhibitor α-methyl-para-tyrosine (Carlsson and Carlsson, 1989 a; Carlsson and Svensson, 1990 b). From this can be inferred that these compounds are capable of stimulating locomotor activity independently of catecholamines.

Furthermore, a dramatic synergism has been shown between catecholaminergic agonists and NMDA antagonists, with regard to locomotor stimulation in monoamine-depleted mice. When dizocilpine, in an ineffective per se dose, is combined with one of the two α_2-adrenergic agonists clonidine (Carlsson and Carlsson, 1989 b) or L-α-methyl-DOPA (Carlsson and Svensson, 1990 b), a marked locomotor stimulation is induced. This synergism is also observed following combination of clonidine with D-CPPene or either of the non-competitive NMDA antagonists ketamine or PCP (Carlsson and Svensson, 1990 a, b).

Recently, Rao et al. (1990 a) have shown that PCP and the PCP-like NMDA antagonists dexoxadrol, ketamine and dizocilpine enhance dopamine (DA) metabolism in rat mesocortical regions; in contrast, the competitive NMDA antagonists CGS-19755 and CPP (3-(2-carboxypiperazine-4-yl)-1-propyl-1-phosphonic acid) do not influence DA turnover. Taken together, the above mentioned data suggest that locomotor stimulation following treatment with non-competitive NMDA antagonists partly is mediated through activation of central catecholaminergic systems, whereas locomotor stimulation following competitive NMDA antagonists is not. The purpose of the present investigation was to compare the PCP-like, non-competitive NMDA antagonist dizocilpine (Wong et al., 1986) to the competitive NMDA antagonist D-CPPene (Aebischer et al., 1989), with regard to both central DA turnover and locomotor stimulatory properties in mice.

Material and methods

Animals

Male albino mice of the NMRI strain weighing 20–30 g were purchased from ALAB, Sollentuna.

Drugs

Dizocilpine ((+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine hydrogen maleate; MK-801; Research Biochemical Inc., MA, USA), D-CPPene (3-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid; SDZ EAA494; generously supplied by Dr. P. L. Herrling at Sandoz Research Institute, Berne) and NSD 1015 (3-hydroxybenzylhydrazine hydrochloride; synthesized by the Organic Chemistry Unit of this department) were dissolved in physiological saline in an ultrasonic bath. Haloperidol (Leo) was dissolved in a few drops of glacial acetic acid and physiological saline. The drugs were injected intraperitoneally (i.p.) in a volume of 10 ml/kg if not otherwise stated.

Biochemical analyses

The animals were killed by decapitation 60 min. following administration of dizocilpine or D-CPPene, if not otherwise stated. In the experiments where aromatic L-amino acid decarboxylase and monoamine oxidase (MAO) were inhibited, NSD 1015 was administered exactly 15 min. before decapitation. After decapitation the brains were immediately taken out and placed on an ice-chilled petri dish. The brains were dissected according to the method described by Carlsson and Lindqvist (1973 a) into the corpus striatum, the limbic forebrain (containing i.a. the nucleus accumbens and olfactory tubercles) and the remaining parts of the hemispheres (essentially cortex). The dissection parts were stored at −70 °C until they were analysed by high performance liquid chromatography with electrochemical detection according to standard principles.

Locomotor registration

Locomotor activity was measured by means of an "M/P 40 Fc Electronic Motility Meter" (Motron Products, Stockholm) with 40 photoconductive sensors (5 rows × 8, centre-centre distance 40 mm), with one animal at a time in the motility meters.

Statistics

Mann-Whitney U-test was used for comparisons of locomotor activity between groups. Biochemical data were analysed by means of ANOVA followed by Fisher's protected least significant difference.

Results

Biochemistry

The effects of the non-competitive NMDA antagonist dizocilpine (0.5, 1, 5 and 10 mg/kg) on concentrations of brain monoamines and monoamine metabolites are shown in Table 1 a. The general pattern was an increase in levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum and the limbic forebrain, as well as in the hemispheres (not shown), although statistical significance was not reached in all doses. There was also a weak increase in the levels of DA in the striatum. Following treatment with 1 mg/kg of dizocilpine there was a significant increase in 5-hydroxyindoleacetic acid (5-HIAA) in the striatum.

Dizocilpine (0.5 mg/kg) was also given to animals treated with NSD 1015, which is a centrally acting inhibitor of aromatic L-amino acid decarboxylase, also called DOPA decarboxylase (Carlsson and Lindqvist, 1973 b). Further-

Table 1 a. Effects of dizocilpine (0.5–10 mg/kg) on concentrations (ng/g) of monoamines and monoamine metabolites in mouse striatum and limbic forebrain. Dizocilpine (20 ml/kg; s.c. in in exp. 2) was administered 60 min. (75 min in exp. 1) before decapitation. Shown are the means ± s.e.m. N = 10 (exp. 1) and 5 (exp. 2). ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs controls

Corpus striatum (concentrations in ng/g)

Treatment	DA	3-MT	DOPAC	HVA	5-HT	5-HIAA
Experiment 1						
NaCl	5225 ± 448	334 ± 31	535 ± 56	928 ± 78	464 ± 38	569 ± 52
Dizocilp. 0.5 mg/kg	5290 ± 427	352 ± 36	662 ± 32	1258 ± 79**	551 ± 48	559 ± 37
Experiment 2						
NaCl	8395 ± 251	415 ± 36	664 ± 26	878 ± 32	451 ± 26	356 ± 16
Dizocilp. 1 mg/kg	9449 ± 435*	362 ± 20	1014 ± 70***	1185 ± 78**	515 ± 22	455 ± 21**
Dizocilp. 5 mg/kg	9593 ± 314*	356 ± 16	975 ± 54***	1128 ± 91	480 ± 21	395 ± 28
Dizocilp. 10 mg/kg	8975 ± 175	361 ± 42	968 ± 23***	1176 ± 59**	460 ± 33	382 ± 22

Limbic forebrain (ng/g)

Treatment	DA	3-MT	DOPAC	HVA	5-HT	5-HIAA
Experiment 1						
NaCl	2191 ± 182	138 ± 14	310 ± 29	438 ± 27	840 ± 54	549 ± 30
Dizocilp. 0.5 mg/kg	2187 ± 125	133 ± 10	449 ± 29**	684 ± 52***	776 ± 33	528 ± 36
Experiment 2						
NaCl	2329 ± 125	80 ± 11	343 ± 18	276 ± 15	884 ± 23	375 ± 17
Dizocilp. 1 mg/kg	2310 ± 116	60 ± 3	507 ± 21***	381 ± 24*	880 ± 22	404 ± 11
Dizocilp. 5 mg/kg	2723 ± 223	76 ± 9	530 ± 29***	438 ± 41***	903 ± 37	387 ± 27
Dizocilp. 10 mg/kg	2289 ± 70	67 ± 13	506 ± 13***	402 ± 16**	883 ± 17	352 ± 10

Table 1 b. Effects of dizocilpine (0.5 mg/kg s.c.) given to animals treated with NSD 1015 (100 mg/kg) on concentrations (ng/g) of DOPA, 5-HTP (5-hydroxytryptophan) and 3-MT in mouse striatum and limbic forebrain. Dizocilpine was administered 60 min. and NSD 1015 15 min. before decapitation. Shown are the means ± s.e.m. N = 10. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs controls

Corpus striatum (concentrations in ng/g)

Treatment	DOPA	3-MT	5-HTP
NaCl	608 ± 32	453 ± 39	95 ± 12
Dizocilp. 0.5 mg/kg	798 ± 39**	600 ± 48*	124 ± 14

Limbic forebrain (ng/g)

Treatment	DOPA	3-MT	5-HTP
NaCl	253 ± 22	79 ± 14	227 ± 39
Dizocilp. 0.5 mg/kg	377 ± 22***	131 ± 20*	222 ± 9

more, NSD 1015 is an MAO inhibitor (Nissbrandt et al., 1988). In Table 1 b it is shown that dizocilpine significantly increased concentrations of 3,4-dihydroxyphenylalanine (DOPA) and 3-methoxytyramine (3-MT) in both the striatum and the limbic forebrain following inhibition of DOPA decarboxylase and MAO.

In Table 2 a the effects of the competitive NMDA receptor antagonist D-CPPene (3, 8, 20 and 40 mg/kg) are shown. Striatal 3-MT levels were significantly decreased in all doses tested. In experiment 2 the animals were killed 100 min. following the administration of D-CPPene, i.e. when maximal locomotor stimulation was observed (not shown). In the limbic forebrain but not in the striatum levels of DOPAC increased, except following the lowest dose. No consistent changes in HVA levels were found. Following the lowest dose of D-CPPene 5-HIAA levels decreased in both the striatum and the limbic forebrain.

When D-CPPene (3 mg/kg) was given to animals treated with NSD 1015, no change was observed in concentrations of DOPA, but there was a tendency towards decreasing concentrations of 3-MT in the striatum (Table 2 b).

Behaviour

Immediately following injections of dizocilpine (0.3 or 0.8 mg/kg) or D-CPPene (3 or 8 mg/kg) the animals were placed in the motility meters and locomotor activity was registered during 80 min. Both doses of dizocilpine caused a significant increase in locomotor activity (Fig. 1).

In contrast, 3 or 8 mg/kg of D-CPPene did not increase spontaneous locomotion (Fig. 1). However, when higher doses of D-CPPene were given and the observation period was extended to 160 min., the locomotor stimulatory

Table 2 a. Effects of D-CPPene (3, 8, 20 and 40 mg/kg) on concentrations (ng/g) of monoamines and monoamine metabolites in mouse striatum and limbic forebrain. D-CPPene was administered 60 min. before decapitation in exp. 1 and 100 min. before decapitation in exp. 2. Shown are the means ± s.e.m. In exp. 1 the sensitivity of the HPLC system did not allow detection of 3-MT in the limbic forebrain. N = 10 (NaCl, exp. 1), 8 (D-CPPene, exp. 1) and 6 (exp. 2). ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs controls

Corpus striatum (concentrations in ng/ng)

Treatment	DA	3-MT	DOPAC	HVA	5-HT	5-HIAA
Experiment 1						
NaCl	8125 ± 474	528 ± 27	793 ± 31	1187 ± 64	354 ± 20	355 ± 15
CPPene 3 mg/kg	8197 ± 551	400 ± 29**	734 ± 92	905 ± 37**	345 ± 33	278 ± 12***
CPPene 8 mg/kg	9123 ± 455	417 ± 35*	938 ± 31	1085 ± 57	364 ± 25	369 ± 14
Experiment 2						
NaCl	6959 ± 203	510 ± 36	694 ± 49	1085 ± 53	323 ± 21	373 ± 29
CPPene 20 mg/kg	7260 ± 183	374 ± 42*	837 ± 65	1115 ± 48	299 ± 11	385 ± 15
CPPene 40 mg/kg	6646 ± 409	343 ± 33**	764 ± 105	1033 ± 81	313 ± 24	354 ± 27

Limbic forebrain (ng/g)

Treatment	DA	3-MT	DOPAC	HVA	5-HT	5-HIAA
Experiment 1						
NaCl	2289 ± 155	–	275 ± 18	397 ± 26	819 ± 20	397 ± 18
CPPene 3 mg/kg	2570 ± 201	–	313 ± 21	371 ± 33	817 ± 38	337 ± 26*
CPPene 8 mg/kg	2382 ± 228	–	360 ± 38*	361 ± 37	800 ± 32	350 ± 16
Experiment 2						
NaCl	2161 ± 218	145 ± 16	278 ± 27	456 ± 35	875 ± 29	431 ± 19
CCPene 20 mg/kg	1989 ± 90	95 ± 10*	393 ± 27*	487 ± 8	814 ± 27	431 ± 21
CCPene 40 mg/kg	2147 ± 262	115 ± 21	405 ± 51*	525 ± 43	830 ± 22	419 ± 41

Table 2 b. Effects of D-CPPene (3 mg/kg) given to animals treated with NSD 1015 (100 mg/kg) on concentrations (ng/g) of DOPA, 5-HTP and 3-MT in mouse striatum and limbic forebrain. D-CPPene was administered 60 min. and NSD 1015 15 min. before decapitation. Shown are the means ± s.e.m. N = 6 (limbic forebrain) and 5 (striatum)

Corpus striatum (concentrations in ng/g)

Treatment	DOPA	3-MT	5-HTP
NaCl	570 ± 41	535 ± 39	75 ± 14
CPPene 3 mg/kg	531 ± 33	449 ± 28	85 ± 6

Limbic forebrain (ng/g)

Treatment	DOPA	3-MT	5-HTP
NaCl	223 ± 26	104 ± 17	172 ± 11
CPPene 3 mg/kg	223 ± 11	95 ± 9	142 ± 7

potential of D-CPPene was revealed (Fig. 2). During the interval 60–160 min. locomotor activity was significantly ($p < 0.05$) enhanced by 20 and 40 mg/kg of D-CPPene vs controls. There was also a significant positive correlation between dose of D-CPPene and motility counts for the interval 60–160 min. ($r = 0.60$, $p = 0.006$). Note that D-CPPene depressed rather than stimulated locomotor activity at the beginning of the observation period; during the interval 0–30 min.,

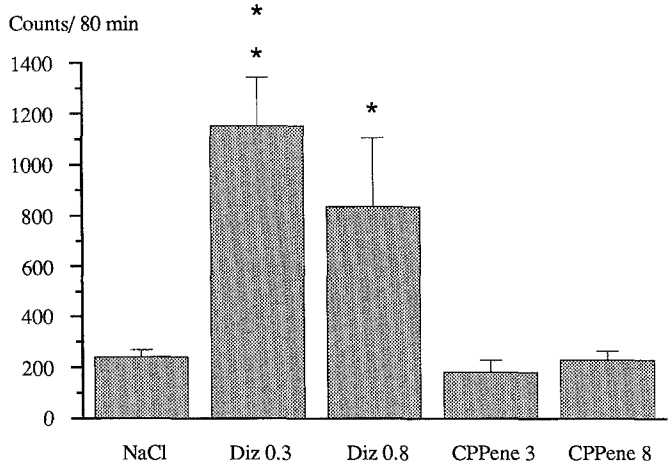

Fig. 1. Effects of dizocilpine and D-CPPene on locomoter activity in mice. Immediately following injections of dizocilpine (0.3 or 0.8 mg/kg) or D-CPPene (3 or 8 mg/kg) the animals were placed in the motility meters and locomotor activity was registered during 80 min. Shown are the means ± s.e.m. N = 6 (NaCl), 5 (dizocilpine 0.3 mg/kg and D-CPPene 8 mg/kg), 4 (D-CPPene 3 mg/kg) and 3 (dizocilpine 0.8 mg/kg). $^{**}p < 0.01$, $^{*}p < 0.05$ vs controls

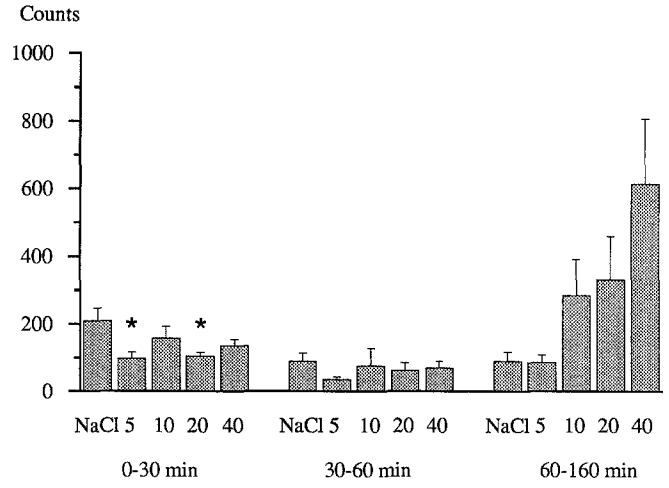

Fig. 2. Effects of various doses of D-CPPene on locomotor activity in mice. Immediately following injections of D-CPPene (5, 10, 20 and 40 mg/kg) the animals were placed in the motility meters and locomotor activity was registered during 160 min. Shown are the means ± s.e.m. for 0–30 min., 30–60 min, and 60–160 min., respectively. N = 5 (4 in the case of D-CPPene 40 mg/kg). There was a significant positive correlation between dose of D-CPPene and motility counts for the interval 60–160 min. ($r = 0.60$, $p = 0.006$). *$p < 0.05$ vs controls

locomotor activity was significantly ($p < 0.05$) *decreased* by 5 and 20 mg/kg of D-CPPene vs controls.

In Fig. 3 the effects of haloperidol pretreatment on D-CPPene-induced lo-

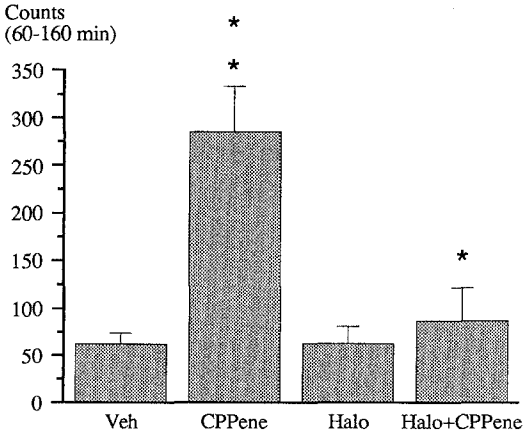

Fig. 3. Effect of haloperidol pretreatment on D-CPPene-induced locomotor activity in mice. Haloperidol (0.1 mg/kg) was administered 30 min. prior to D-CPPene treatment. Immediately following injection of D-CPPene (20 mg/kg) the animals were placed in the motility meters and locomotor activity was registered during 160 min. Shown are the means ± s.e.m. for the period 60–160 min. N = 6. **$p < 0.01$ vs vehicle, *$p < 0.05$ vs D-CPPene

comotor stimulation are shown. Haloperidol (0.1 mg/kg) was given 30 min. prior to administration of D-CPPene. The animals were placed in the motility meters immediately following injections of D-CPPene (20 mg/kg) and locomotor activity was registered during 160 min. The low dose of haloperidol used in this experiment did not influence the behaviour of normal, habituated animals. However, the locomotor stimulation produced by D-CPPene was almost completely antagonized.

Discussion

Levels of DOPAC reflect DA synthesis and metabolism within the nerve terminal. The principal source of HVA is O-methylation by catechol-O-methyltransferase (COMT) of DOPAC, effluxed from the nerve terminal; only lesser amounts of HVA are derived from deamination of 3-MT by MAO (Westerink and Korf, 1976). Consequently, in most cases DOPAC and HVA change in parallel. The only index of the amount of DA *released* from the nerve terminals is the O-methylated DA metabolite 3-MT (Carlsson and Lindqvist, 1963; Kehr, 1980), which is formed by COMT, an enzyme located exclusively outside the nerve terminal (Carlsson and Hillarp, 1962); see also Wood and Altar (1988).

Dizocilpine increased concentrations of DOPAC and HVA in the striatum and the limbic forebrain, as well as in the hemispheres, whereas DA concentrations were unchanged or slightly increased. When dizocilpine was given to animals where DOPA decarboxylase and MAO were inhibited by means of NSD 1015, an increase in levels of DOPA and 3-MT were observed in both the striatum and the limbic forebrain. Taken together, these findings suggest that dizocilpine stimulates synthesis and release of DA in mouse brain. Recently it was shown that systemic administration of the non-competitive NMDA antagonists dizocilpine, PCP and ketamine to *rats* increased DOPAC levels in mesocortical regions and in the striatum. However, no changes in DOPAC levels were observed in the nucleus accumbens and cingulate cortex (Rao et al., 1990 a, b). Enhanced DA release following perfusion of *rat* nucleus caudatus and accumbens with dizocilpine has been shown by means of microdialysis technique (Imperato et al., 1990).

Following dizocilpine treatment the animals displayed a clear-cut locomotor stimulation. 0.3 mg/kg of dizocilpine was more efficacious than 0.8 mg/kg. Following the lower dose the animals displayed a varied pattern of locomotion and a low degree of stereotypies, whereas the larger dose induced predominantly stereotypies, the animals sitting at the same spot swaying there and back with the anterior part of the body, most of the time.

In a recent study it was shown that dizocilpine-induced locomotion and sniffing stereotypies in *rats*, were partially blocked by haloperidol (Tiedtke et al., 1990), a DA antagonist with α_1-adrenoceptor antagonistic properties (Hyttel et al., 1985), thus suggesting involvement of a dopaminergic and/or an adrenergic mechanism. Early observations of Clineschmidt et al. (1982 a, b) also indicate involvement of a catecholaminergic mechanism, since both locomotor

stimulation and anticonvulsant activity of dizocilpine in mice partially were blocked by haloperidol or the α_1-adrenergic antagonist prazosin. Furthermore, dizocilpine-induced ipsiversive turning in 6-hydroxydopamine lesioned rats was antagonized by the α_1-adrenergic antagonists aceperone, azapetine and prazosin as well as by noradrenaline synthesis inhibition by means of FLA-63, a DA-β-hydroxylase inhibitor (Martin and Papp, 1984).

In contrast to the clear-cut increase in DA turnover following dizocilpine treatment, only slight changes in levels of DA metabolites were observed following D-CPPene treatment. It is interesting to note that striatal 3-MT levels, measured in brains of animals decapitated when the locomotor stimulation was maximal, were decreased. These data suggest that dizocilpine and D-CPPene may have opposite effects on DA release. An uncertain factor, however, is our failure to demonstrate differences in the rate of DA disappearance following treatment with these compounds. In several experiments where tyrosine hydroxylase, the rate limiting enzyme in DA synthesis, was inhibited by means of the competitive inhibitor α-methyl-para-tyrosine, neither dizocilpine nor D-CPPene influenced the rate of DA disappearance.

Following D-CPPene treatment in lower doses no locomotor stimulation was observed, only ataxia. However, in larger doses locomotor stimulation was observed, approximately 60 min. after administration. The gross appearance of these animals was very similar to that produced by dizocilpine, i.e. a combination of stereotypies and a varied pattern of locomotion, with predominantly stereotypies in the largest dose.

The D-CPPene-induced locomotor stimulation, but not the stereotypic side-to-side head-weaving behaviour, was almost completely antagonized by pretreatment with a low dose of haloperidol, indicating an involvement of catecholaminergic neurons. However, the decreased levels of 3-MT following D-CPPene treatment suggest that the locomotor stimulatory effects are not mediated via release of DA; hence, the catecholaminergic system seems to play primarily a permissive role in this context. Our experiments with monamine-depleted mice suggest that important interactions between the glutamatergic and catecholaminergic neurons occur on a level that is *postsynaptic* in relation to these neurons; in monoamine-depleted mice a forceful synergism is observed when a catecholaminergic agonist is combined with an NMDA antagonist. In line with this, Girault et al. (1990) have recently found that the activity of protein phosphatase-1 in striatonigral neurones is decreased by DA receptor stimulation and increased by NMDA receptor stimulation, suggesting that stimulation of postsynaptic DA receptors may result in a facilitation of the effects of an NMDA antagonist.

In contrast to the present data Imperato et al. (1990) observed an increased DA release following local perfusion of *rat* nucleus caudatus and accumbens with D-CPPene, and this DA release was accompanied by strong behavioral activation. The discrepancy in the case of DA release between our findings and those of Imperato et al., may be attributed to species differences or drug ad-

ministration route. In this context it is interesting to note that following intracerebroventricular injection to rat of the competitive NMDA antagonist CPP, hyperactivity and ataxia was observed. When CPP was injected into the frontal cortex, the rats showed an episodic darting behavior without ataxia (O'Neill and Liebman, 1987).

When given to mice with intact monoaminergic systems, dizocilpine has locomotor stimulatory properties only in doses *smaller* than 1 mg/kg i.p., with a maximum response at 0.5 mg/kg (J. Engel, 1990; unpublished observations). The effects of dizocilpine on intact animals are probably mediated both via release of catecholamines and by suppression of glutamatergic transmission, both effects mediated by NMDA antagonism. When dizocilpine is given to monoamine-depleted animals the locomotor stimulation arises from suppression of glutamatergic transmission only, which may be the reason why a larger dose (>1 mg/kg) is required. However, when dizocilpone is combined with an α_2-adrenergic agonist, like clonidine or L-α-methyl-DOPA, or a subthreshold dose of a mixed dopaminergic agonist, like apomorphine, a smaller dose of dizocilpine is sufficient (Carlsson and Carlsson, 1989 a, b; Carlsson and Svensson, 1990 b). A synergistic effect was also observed when clonidine was combined with either ketamine (Carlsson and Svensson, 1990 a), PCP or D-CPPene (Carlsson and Svensson, 1990 b). The gross behavioral appearances produced by these combinations were almost indistinguishable from each other, suggesting that the same mechanism is responsible for the stimulation observed.

The postsynaptic effects of dizocilpine and D-CPPene may be similar, but there are evidently differences in the effects on catecholaminergic neurons. Which mechanisms are then responsible for the differences in DA turnover? Rao et al. (1990) have recently proposed that a PCP binding site functionally uncoupled to NMDA receptors, is responsible for the stimulation of DA turnover following treatment with PCP-like, non-competitive NMDA antagonists, which does not occur following the competitive NMDA antagonists CPP and CGS-19755. But against this hypothesis speak a large number of observations, suggesting a tight morphological and functional coupling between the PCP binding site and the NMDA receptor complex (see Maragos et al., 1988).

Heterogeneity of the NMDA receptor population is another tentative explanation, which does not require the existence of PCP binding sites uncoupled to NMDA receptors. According to this hypothesis some NMDA receptors are functionally coupled to catecholaminergic systems, stimulating or inhibiting transmitter synthesis and release, whereas other subtypes of NMDA receptors are functionally uncoupled to catecholamines. A heterogeneity of NMDA receptors may also underlie the relative separation between anticonvulsant activity and motor effects of the competitive NMDA antagonist CGS-19755. Antagonism of NMDA-induced convulsions in mice has an ED_{50} of 2.1 mg/kg i.p. but locomotor stimulation does not occur in smaller doses than 30 mg/kg (Bennet et al., 1989). In contrast, most non-competitive NMDA antagonists, in-

cluding dizocilpine, antagonize NMDA-induced convulsions only in doses higher than those associated with motor effects (Willets et al., 1990).

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References

Aebischer B, Frey P, Haerter HP, Herrling PL, Mueller W, Olverman HJ, Watkins JC (1989) Synthesis and NMDA antagonistic properties of the enantiomers of 4-(3-phosphonopropyl)piperazine-2-carboxylic acid (CPP) and of the unsaturated analogue (E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (CPPene). Helv Chim Acta 72: 1043–1051

Bennet DA, Bernard PS, Amrick CL, Wilson DE, Liebman JM, Hutchison AJ (1989) Behavioral pharmacological profile of CGS 19755, a competitive antagonist at N-methyl-D-aspartate receptors. J Pharmacol Exp Ther 250: 454–460

Carlsson A, Hillarp NE (1962) Formation of phenolic acids in brain after administration of 3,4-dihydroxyphenylalanine. Acta Physiol Scand 55: 95–100

Carlsson A, Lindqvist M (1963) Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol Toxicol 20: 140–144

Carlsson A, Lindqvist M (1973 a) Effect of ethanol on the hydroxylation of tyrosine in rat brain in vivo. J Pharm Pharmacol 25: 437–440

Carlsson A, Lindqvist M (1973 b) In vivo measurements of tryptophan and tyrosine hydroxylase activities in mouse brain. J Neural Transm 34: 79–91

Carlsson M, Carlsson A (1989 a) The NMDA antagonist MK-801 causes marked locomotor stimulation in monoamine-depleted mice. J Neural Transm 75: 221–226

Carlsson M, Carlsson A (1989 b) Dramatic synergism between MK-801 and clonidine with respect to locomotor stimulatory effect in monoamine-depleted mice. J Neural Transm 77: 65–71

Carlsson M, Svensson A (1990 a) Interfering with glutamatergic neurotransmission by means of NMDA antagonist administration discloses the stimulatory potential of other transmitter systems. Pharmacol Biochem Behav 36: 45–50

Carlsson M, Svensson A (1990 b) The non-competitive NMDA antagonists MK-801 and PCP, as well as the competitive NMDA antagonist SDZ EAA494 (D-CPPene), interact synergistically with clonidine to promote locomotion in monoamine-depleted mice. Life Sci 47: 1729–1736

Clineschmidt BV, Martin GE, Bunting PR, Papp NL (1982 a) Central sympathomimetic activity of (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetric and apparent anxiolytic properties. Drug Dev Res 2: 135–145

Clineschmidt BV, Martin GE, Bunting PR (1982 b) Anticonvulsant activity of (+)-5-methyl-10,11-diydro-5H-dibenzo(a,d)cycloheptan-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic and apparent anxiolytic properties. Drug Dev Res 2: 135–145

Girault J-A, Halpain S, Greengard P (1990) Excitatory amino acid antagonists and Parkinson's disease. Trends Pharmacol Sci 13: 325–326

Hyttel J, Larsen J-J, Christensen AV, Arnt J (1985) Receptor-binding profiles of neuroleptics. In: Casey DE, Chase TN, Christensen AV, Gerlach J (eds) Dyskinesia – research and treatment. Springer, Berlin Heidelberg New York Tokyo (Psychopharmacology [Suppl 2]: 9–18)

Imperato A, Scrocco MG, Bacchi S, Angelucci L (1990) NMDA receptors and in vivo dopamine release in the nucleus accumbens and caudatus. Eur J Pharmacol 187: 555–556

Kehr W (1981) 3-Methoxytyramine and normetanephrine as indicators of dopamine and noradrenaline release in mouse brain in vivo. J Neural Transm 50: 165–178

Maragos WF, Penney JB, Young AB (1988) Anatomic correlation of NMDA and ^{3}H-TCP-labeled receptors in rat brain. J Neurosci 8: 493–501

Martin GE, Papp NL (1984) Blockade of MK-801 induced ipsiversive turning in 6-OHDA lesioned rats by α_1-adrenoceptor antagonists. Pharmacol Biochem Behav 20: 893–897

O'Neill KA, Liebman JM (1987) Unique behavioral effects of the NMDA antagonist, CPP, upon injection into the medial pre-frontal cortex of rats. Brain Res 435: 371–376

Nissbrandt H, Engberg G, Wikström H, Magnusson T, Carlsson A (1988) NSD 1034: an amino acid decarboxylase inhibitor with a stimulatory action on dopamine synthesis not mediated by classical dopamine receptors. Naunyn-Schmiedebergs Arch Pharmacol 338: 148–161

Rao TS, Kim HS, Lehmann J, Martin LL, Wood PL (1990 a) Selective activation of dopaminergic pathways in the mesocortex by compounds that act at the phencyclidine (PCP) binding site: tentative evidence for PCP recognition sites not coupled to N-methyl-D-aspartate (NMDA) receptors. Neuropharmacology 29: 225–230

Rao TS, Kim HS, Lehmann J, Martin LL, Wood PL (1990 b) Interactions of phencyclidine receptor agonist MK-801 with dopaminergic system: regional studies in the rat. J Neurochem 54: 1157–1162

Schmidt WJ (1986) Intrastriatal injection of DL-2-amino-5-phosphonovaleric acid (AP-5) induces sniffing stereotypy that is antagonized by haloperidol and clozapine. Psychopharmacology 90: 123–130

Tiedtke PI, Bischoff C, Schmidt WJ (1990) MK-801-induced stereotypy and its antagonism by neuroleptic drugs. J Neural Transm 81: 173–182

Westerink BHC, Korf J (1976) Turnover of acid dopamine metabolites in striatal and mesolimbic tissue of the rat brain. Eur J Pharmacol 37: 249–255

Willets J, Balster RL, Leander JD (1990) The behavioral pharmacology of NMDA receptor antagonists. Trends Pharmacol Sci 11: 423–428

Wong EHF, Kemp JA, Priestly T, Knight AR, Woodruff GN, Iversen LL (1986) The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. Proc Natl Acad Sci USA 83: 7104–7108

Wood PL, Altar A (1988) Dopamine release in vivo from nigrostriatal, mesolimbic and mesocortical neurons: utility of 3-methoxytyramine measurements. Pharmacol Rev 40: 164–187

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