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Blockade of NMDA receptors in the nucleus accumbens elicits spontaneous tail-flicks in rats

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Abstract

The open channel blocker at *N*-methyl-D-aspartate (NMDA) receptors, dizocilpine, stereospecifically elicited spontaneous tail-flicks in rats — a reaction similar to those elicited by other drugs (tenocyclidine, phencyclidine and ketamine) acting as open channel blockers. Their relative potencies were strongly correlated with affinities at NMDA binding sites and labeled by [^{3}H]dizocilpine in the frontal cortex ($r = 0.94$) and, as determined previously [Millan, M.J., Seguin, L., 1994. Chemically-diverse ligands at the glycine B site coupled to *N*-methyl-D-aspartate (NMDA) receptors selectively block the late phase of formalin-induced pain in mice, Neurosci. Lett., 178 (1994) 139–143], potency for eliciting antinociception (0.93). The competitive antagonists at the NMDA receptor recognition site, (±)3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), 4-phosphonomethyl-2-piperidine carboxylic acid (CGS19755), D,L-(*E*)-2-amino-4-methylphosphono-3-pentanoic acid (CGP37849) and (3*E*)-1-ethyl ester-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP39551), likewise dose-dependently evoked spontaneous tail-flick. In contrast, antagonists/weak partial agonists at the coupled, glycine B site, 7-chloro-4-hydroxy-3-(3-phenoxy) phenyl-2(*H*)-quinolinone (L701,324), (+)-1-hydroxy-3-aminopyrrolidine-2-one ((+)-HA966), (3*R*,4*R*)-3-amino-1-hydroxy-4-methyl-2-pyrrolidinone (L687,414), 6,7-dichloro-1, 4-dihydro-5-nitro, 2,3 quinoxalinedione (ACEA1021) and 2-carboxy-4,6-dichloro (1*H*)-indole-3-propanoic acid (MDL29,951), were inactive. NMDA abolished induction of spontaneous tail-flick by CPP and CGS19755, but not by dizocilpine. Upon bilateral injection into the nucleus accumbens, dizocilpine immediately and dose-dependently elicited spontaneous tail-flick, but it was ineffective in the ventrotegmental area and striatum. Similarly, injection of CPP into the nucleus accumbens elicited spontaneous tail-flick. Neither dizocilpine nor CPP elicited spontaneous tail-flick upon administration onto lumbar spinal cord. In conclusion, a pharmacologically specific spontaneous tail-flick-response is elicited by both open channel blockers and recognition site antagonists, but not glycine B site antagonists, at NMDA receptors. Their actions, mediated in the nucleus accumbens, may be differentiated by their respective resistance and sensitivity to NMDA.

Keywords: NMDA receptors; Glutamate; Dizocilpine; Nucleus accumbens; Tail-flick

1. Introduction

Excitatory amino acids exert their actions via a variety of ionotropic and metabotropic receptor types (Collingridge and Lester, 1989; Pin and Duvoisin, 1995; Ozawa et al., 1998). Of these, *N*-methyl-D-aspartate (NMDA) receptors continue to attract considerable interest in view of their broad functional implication in the control of mood, cognition, motor behaviour, nociception and other functions (Parsons et al., 1998). NMDA receptors possess several sites via which the activity of the associated cation-permeable channel may be modulated. Within the ion channel itself, there exists a site highly sensitive to magnesium, which interrupts the passage of ions through the channel (Sharma and Stevens, 1996; Parsons et al., 1998). This site underlies the relative quiescence of NMDA receptors in the absence of neuronal depolarization mediated by, for example, activation of ionotropic α-amino-3-

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hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors — which display rapid kinetics (Bleakman and Lodge, 1998; Ozawa et al., 1998; Millan, 1999). An additional site localized within the channel is accessed by open channel blockers, such as the dissociative anesthetics, ketamine and phencyclidine, as well as by the structurally related analogue, tenocyclidine, and the cycloheptene derivative, dizocilpine, of which the (+)-isomer is some 10-fold more potent than its (−)-counterpart. Although ketamine and phencyclidine act via other mechanisms, such as σ receptors and monoamine uptake sites (Gorelick and Balster, 1995; Steinpreis, 1996; Millan et al., in press a), the pronounced selectivity of dizocilpine for NMDA receptors has rendered it important as a tool for exploration of their pathophysiological significance. Indeed, dizocilpine manifests potent antinociceptive, anticonvulsive and other functional properties in vivo (Dickenson, 1997; Ozawa et al., 1998; Parsons et al., 1998; Millan, 1999).

At the recognition site for NMDA, glutamate and other excitatory amino acids, several ligands behave as competitive antagonists. Such drugs include the structurally related CPP (4-phosphonomethyl-2-piperidine carboxylic acid) and CGS19755 (4-phosphonomethyl-2-piperidine carboxylic acid), as well as the phosphono-amino acid derivative, CGP37849 (D,L-(*E*)-2-amino-4-methylphosphono-3-pentanoic acid), and its carboxyethylester, CGP39551 ((3*E*)-1-ethyl ester-2-amino-4-methyl-5-phosphono-3-pentenoic acid) (Bennett et al., 1989; Schmutz et al., 1990). Like the open channel blockers, their ability to occupy NMDA receptor recognition sites upon systemic administration to rodents is reflected in antinociceptive, anticonvulsive and other functional actions (Schmutz et al., 1990; Löscher and Hönack, 1991; Millan and Seguin, 1994; Dickenson, 1997; Parsons et al., 1998). In addition to the NMDA receptor recognition site, a ''glycine B'' site is positively coupled to the associated ion channel and its activation may be mandatory for full operation of the NMDA receptor complex — although it is controversial as to whether this site is invariably saturated by [physiological concentrations of] endogenous glycine in vivo (Wood, 1995; Berger et al., 1998; Viu et al., 1998). In any case, chemically diverse and selective glycine B receptor antagonists/weak partial agonists, such as L701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(*H*)-quinolinone), L687,414 ((3*R*,4*R*)-3-amino-1-hydroxy-4-methyl-,2-pyrrolidinone), MDL29,951 (2-carboxy-4,6-dichloro (1*H*)-indole-3-propanoic acid), ACEA1021 (6,7-dichloro-1,4-dihydro-5-nitro, 2,3 quinoxalinedione) and (+)-HA966 ((+)-1-hydroxy-3-aminopyrrolidine-2-one), exert marked antinociceptive properties in rodents (Henderson et al., 1990; Baron et al., 1992; Foster et al., 1992; Millan and Seguin, 1994; Keana et al., 1995; Bristow et al., 1996; Dickenson, 1997).

The high density of NMDA receptors in the ventral horn, basal ganglia, nucleus accumbens and frontal cortex provides a neuroanatomical substrate for the above-mentioned role of NMDA receptors in the control of motor behaviour (Meltzer et al., 1997; Morari et al., 1998; Millan, 1999). This is typified, for open channel blockers such as dizocilpine, by stereotypic and motor-stimulant effects at low doses, and motor discoordination and ataxia at higher doses (Tricklebank et al., 1989; Carlsson and Carlsson, 1990; Parsons et al., 1998). Together with their psychotomimetic properties, such motor actions compromise the potential clinical utility of open channel blockers and recognition site antagonists at NMDA receptors, although the therapeutic window of the latter may be somewhat greater (Tricklebank et al., 1989; Leander, 1992; Parsons et al., 1998). Recently, it has been suggested that the security profile of glycine B receptor antagonists may be superior to those of both open channel blockers and NMDA receptor recognition site antagonists (Hargreaves et al., 1993; Bristow et al., 1996; Parsons et al., 1998; Witkin et al., 1997).

We have identified a novel and simple behavioural response elicited by systemic administration of racemic dizocilpine. That is, spontaneous tail-flicks elicited in the apparent absence of extraneous, sensory stimulation (Millan, 1991). This response was mimicked by the NMDA receptor recognition site antagonist, CPP, but not by the glycine B ligand, (+)-HA966, nor by drugs acting at polyamine sites or other potential modulatory sites of the NMDA receptor complex (Millan, 1991). Moreover, AMPA/kainate receptor antagonists and a broad range of pharmacologically diverse motor stimulant drugs are likewise ineffective in provoking spontaneous tail-flick: notably, catecholamine releasers/uptake inhibitors, such as cocaine; direct dopamine receptor agonists, such as apomorphine; μ-opioids such as morphine; hallucinogens such as mescaline; GABA receptor antagonists, such as bicucculine; adenosine receptor antagonists, such as caffeine and muscarinic receptor antagonists, such as scopolamine (Millan, 1991; Millan et al., 1991). These observations suggest that spontaneous tail-flick may provide a useful model for the detection and characterization of drug activity at NMDA receptors in vivo.

This possibility was systematically evaluated herein. In order to underpin the specificity of actions of open channel blockers, their potencies in eliciting spontaneous tail-flick were compared to their affinities at cerebral populations of NMDA receptors, as determined by the displacement of [^{3}H]dizocilpine binding. Moreover, we compared the present data to our previously documented reports of their potencies in evoking antinociception and ataxia, prototypical responses to open channel blockers (Dickenson, 1997; Millan, 1999; Millan and Seguin, 1994). Active doses of antagonists at the NMDA receptor recognition site (and glycine B receptor antagonists) were also compared to their previously documented antinociceptive and ataxic actions (Millan and Seguin, 1994). In a complementary set of experiments, employing a microinjection approach, we attempted to localize the population of NMDA receptors mediating spontaneous tail-flicks.

2. Methods

2.1. Measurement and definition of spontaneous tail-flick

Male Wistar rats of 220–230 g (Iffa Credo, L'Arbresle, France) were housed in sawdust-lined cages with unrestricted access to rat chow and water. There was a 12 h/12 h light/dark cycle with lights on/off at 0700/1900. All experiments were undertaken in the light phase. Spontaneous tail-flick were determined exactly as detailed previously (Millan, 1991; Millan et al., 1991; Bervoets et al., 1993) in rats loosely restrained in horizontal, opaque, plastic cylinders with the tail emerging from the back to hang over the edge of the bench. One spontaneous tail-flick was defined as the elevation of the tail to a level higher than that of the body axis. The number of spontaneous tail-flicks emitted was recorded over 5 min. For studies of the influence of systemic drug administration, there was a 5-min adaptation period to the cylinder prior to the recording of spontaneous tail-flicks. For studies of the influence of local drug microinjection, spontaneous tail-flicks were recorded for 5 min immediately following introduction into the cylinder, since it was important not to miss a potential, immediate drug-response.

2.2. Dose–response and time–response relationships for induction of spontaneous tail-flicks by systemic drug administration

The action of systemic administration of drugs was determined 30 min following their administration, with the exception of the antagonists at the NMDA receptor recognition site, CPP, CGS19755, CGP37849 and CGP39551, which were evaluated at 60 min. This later time was based upon preliminary studies of their time-course-of-action and literature studies showing that these lipophobic ligands have a more pronounced delay to peak effect than the other drug classes employed herein. In this regard, a complete time-course-of-action was performed for dizocilpine as compared to CPP, whereby the induction of spontaneous tail-flicks over 5-min periods was evaluated from 0–5 to 240–245 min following their administration. Separate groups of rats were examined at each time-point.

2.3. Influence of NMDA upon induction of spontaneous tail-flicks by open channel blockers and antagonists at the NMDA receptor recognition site

Rats were treated with vehicle or NMDA (40.0 mg/kg, s.c), 15 min prior to administration of vehicle, CPP, CGS19755 or dizocilpine. Sixty minutes after vehicle, CPP or CGS19755, and 30 min after vehicle or dizocilpine, spontaneous tail-flicks were recorded.

2.4. Local drug administration

Bilateral cannulation of the nucleus accumbens, striatum or ventrotegmental area was performed under pentobarbital anaesthesia employing conventional procedures. The coordinates according to the atlas of Paxinos and Watson (1986) were as follows: accumbens (AP = +1.4, $L = \pm 2.0$, DV = −5.8); striatum (AP = +0.45, $L = \pm 2.8$, DV = −3.2) and ventral tegmental area (AP = −4.8, $L = \pm 0.8$, DV = −8.0). One week was allowed for recovery following implantation of guide cannulae. Drugs were injected bilaterally in a volume of 1 μl. Immediately following injection of drugs into the various structures, spontaneous tail-flicks were recorded. All cerebral cannulae positions were verified by conventional histological techniques and data acquired from inappropriate placements were discarded. For intrathecal injections, the technique employed was as described previously (Bervoets et al., 1993). Briefly, rats were anaesthetised with pentobarbital (40.0 mg/kg, i.p.), placed in a stereotaxic apparatus, and a polyethylene catheter (PE-10 of 0.28 mm internal diameter) was inserted caudally into the subarachnoid space through a puncture of the atlanto-occipital membrane. The catheter tip was situated in the lumbar region of the spinal cord. Immediately, as well as 24 h, after placement, the catheters were flushed with their own volume of sterile saline. Animals were tested 1 week after surgery. Drugs were infused in a volume of 10 μl at a speed of 1 μl/3 s. For verification of catheter placement, 10 μl of blue methylene was injected via the catheter, and the tip of catheter localized by dissection of the spinal cord.

2.5. Binding studies

Binding experiments were performed essentially as previously (Yoneda et al., 1990; Cordi et al., 1999) employing the buffer: Tris-acetate 50 mM pH 7.4, Triton 0.08%. NMDA sites were labeled with [^{3}H]MK 801 (1 nM) and non-specific binding was defined with phencyclidine 10 μM. The duration of incubation was 120 min at 22°C. Assays were terminated by filtration through 0.1% polyethylenimine-pretreated Whatman GF/B glass fiber filters washed with ice-cold binding buffer. Competition binding data were analyzed by nonlinear regression to yield inhibitory concentrations$_{50}$ (IC_{50}).

2.6. Drugs

For systemic administration, all drugs were dissolved in sterile water, plus a few drops of lactic acid if necessary, and the pH adjusted to asclose to neutrality as possible (pH > 5.0) with sodium hydroxide. Drugswere injected s.c. in a volume of 1 ml/kg body weight, unless otherwise indicated. For intracerebral and intrathecal microinjection, drugs were dissolved in sterile saline and administered in a

volume of 1 μl. Doses indicated refer to the base. Drug salts, structures, and sources were as follows: (+)-dizocilpine maleate, (−)-dizocilpine maleate, ketamine HCl, phencyclidine HCl and tenocyclidine HCl (Sigma, St. Quentin-Fallavier, France); (±)-CPP = (±)3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, (+)-HA966 = (+)-(1-Hydroxy-3-aminopyrrolidine-2-one) base and MDL29,551 = 2-carboxy-4,6-dichloro (1*H*)-indole-3-propanoic acid (Tocris-Cookson, Bristol, UK); CGP37849 = D,L-(*E*)-2-amino-4-methylphosphono-3-pentanoic acid and CGP39551 = (3*E*)-1-ethyl ester-2-amino-4-methyl-5-phosphono-, 3-pentenoic acid (Ciba-Geigy Laboratories, Basel, Switzerland); ACEA1021 = 6,7-dichloro-1, 4-dihydro-5-nitro, 2,3 quinoxalinedione base, CGS19755 = 4-phosphonomethyl-2-piperidine carboxylic acid, L687,414 = (3*R*,4*R*)-3-amino-1-hydroxy-4-methyl-, 2-pyrrolidinone hemi-D-tartrate, L701,324 = 7-chloro-4-hydroxy-3-(3-phenoxy) phenyl-2(*H*)-quinolinone base and (±)-dizocilpine oxalate were synthesised by Servier chemists (A. Cordi).

2.7. Statistics

Data were analysed by analysis of variance (ANOVA) followed by a post-hoc Dunnett's or Newman–Keuls test as appropriate. The level of significance was set at $P < 0.05$. Pearson Product–Moment correlation coefficients were determined (Fig 2).

3. Results

3.1. Displacement of [^{3}H]dizocilpine binding by open channel blockers

Racemic dizocilpine potently displaced the binding of [^{3}H]dizocilpine to NMDA receptors localized in rat frontal cortex (Table 1). This activity was expressed preferentially by its (+)- as compared to (−)-isomer, the former of which was eight-fold more potent than the latter (Table 1). Two further open channel blockers, tenocyclidine and phencyclidine, likewise showed marked affinity for dizocilpine binding sites, whereas ketamine was weakly active.

3.2. Induction of spontaneous tail-flicks by systemic administration of open channel blockers

The s.c. administration of racemic dizocilpine dose-dependently elicited spontaneous tail-flicks over a dose-range of 0.01–0.16 mg/kg, s.c (Fig. 1). However, its action was expressed biphasically with the dose–response curve inflecting at a dose of 0.16 (Fig. 1). The (+)-isomer of dizocilpine shared this effect in likewise displaying a markedly biphasic dose–response curve for induction of spontaneous tail-flicks (Fig. 1). In accordance with its lower affinity at NMDA binding sites, the (−)-isomer of dizocilpine was about eight-fold less potent than the (+)-isomer (Fig. 1). The action of dizocilpine was expressed rapidly following its s.c. administration, peaking at 30 min (Fig. 1). Several other drugs acting as open channel blockers at NMDA receptors, tenocyclidine, phencyclidine and ketamine, similarly elicited spontaneous tail-flicks in a biphasic fashion (Table 1). For comparative purposes, Table 1 also indicates the potency of these open channel blockers to elicit antinociception (in the formalin test) and ataxia (in the rotarod test), as described previously in Millan and Seguin (1994). In Fig. 2, it is shown that the potency for induction of spontaneous tail-flicks by open channel blockers correlated significantly with their affinity at NMDA receptors, potency of induction of antinociception and potency for induction of ataxia in each case.

3.3. Induction of spontaneous tail-flicks by systemic administration of antagonists at the NMDA receptor recognition site

As illustrated in Fig. 3 and Table 2, the NMDA receptor recognition site antagonists, CPP, CGS19755, CGP37849 and CGP39551, dose-dependently elicited spontaneous tail-flicks. In contrast to open channel blockers, their

Table 1
Affinities of open channel blockers and their potency for induction of STFs as compared to their potencies in eliciting antinociception and ataxia
Affinities are means ± S.E.M. of at least three independent determinations performed in triplicate and expressed as pIC_{50} for displacement of the specific binding of [^{3}H]dizocilpine to NMDA receptors in rat frontal cortex. STFs = spontaneous tail-flicks, expressed as minimal effective dose (MED) ($P < 0.05$ to vehicle in Dunnett's test) and maximal observed effect (MOE) ± S.E.M. NT = Not tested.

Drug	Affinity	STFs		Antinociception[a]	Ataxia[b]
	pIC_{50}	MED	MOE ± S.E.M./5 min (dose)	ID_{50}	ID_{50}
(+)-Dizocilpine	8.63 ± 0.18	0.08	49.1 ± 9.1 (0.08)	0.03	0.08
(±)-Dizocilpine	8.38 ± 0.06	0.16	34.7 ± 6.2 (0.16)	0.15	0.07
(−)-Dizocilpine	7.89 ± 0.12	0.63	31.6 ± 7.1 (0.63)	0.09	0.30
Tenocyclidine	7.89 ± 0.06	0.63	39.5 ± 9.9 (0.63)	NT	1.9
Phencyclidine	7.38 ± 0.12	5.0	34.1 ± 10.6 (5.0)	1.2	1.8
Ketamine	6.16 ± 0.01	10.0	30.3 ± 9.7 (20.0)	7.7	37.0

[a]Antinociception = Inhibitory $dose_{50}$ (ID_{50}) for inhibition of the late phase of licking elicited by plantar injection of formalin to mice (Millan and Seguin, 1994).
[b]Ataxia in mice = ID_{50} for reduction of latency to fall from a rotarod (Millan and Seguin, 1994).

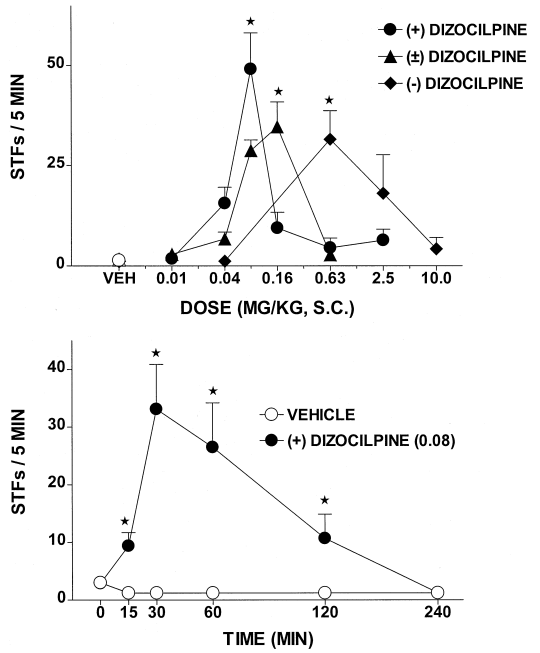

Fig. 1. Stereospecific induction of spontaneous tail-flicks by systemic administration of dizocilpine. Data are means ± S.E.M. $N \geq 5$ per value. The upper panel indicates the dose–response relationship, and the lower panel shows the time-course-of-action of (+)-dizocilpine (0.08 mg/kg, s.c.). ANOVA as follows: Upper panel: (±)-dizocilpine, $F(5,69) = 9.0$, $P < 0.001$; (+)-dizocilpine, $F(6,49) = 12.1$, $P < 0.001$ and (−)-dizocilpine, $F(4,23) = 3.3$, $P < 0.05$. Lower panel: dizocilpine, $F(5,40) = 9.7$, $P < 0.05$ and drug × time, $F(5, 50) = 3.3$, $P < 0.05$. Asterisks indicate significance of difference to corresponding vehicle values in Dunnett's test $^{*}P < 0.05$.

dose–response curves were monophasic up to the highest doses tested. (However, take note that these maximal doses were limited by drug solubility and drug availability.) The time-course for induction of spontaneous tail-flicks by CPP was more protracted than that of dizocilpine and its onset of action slower with a peak effect at 90 min (Fig. 3). As indicated in Table 2, and determined elsewhere (Millan and Seguin, 1994), these antagonists at the NMDA receptor recognition site also elicit antinociception and ataxia. Indeed, the relative potency of these drugs in eliciting spontaneous tail-flicks corresponded well with their relative potency in eliciting antinociception. Notably, for each parameter, CGP37849 and CGP39551, were the most and least potent drugs, respectively (Table 2).

3.4. Lack of induction of spontaneous tail-flicks by glycine B site antagonists

The selective antagonists (or weak partial agonists) at glycine B receptors, L701,324, MDL29,951, ACEA1021, L687,414 and (+)-HA966, did not elicit spontaneous tail-flicks (Table 3), even at doses markedly higher than those previously shown to elicit antinociception (Millan and Seguin, 1994).

3.5. Inhibition of spontaneous tail-flicks elicited by antagonists at the NMDA receptor recognition site with NMDA

NMDA significantly and dose-dependently inhibited the induction of spontaneous tail-flicks by CPP (Fig. 4). It also attenuated the action of CGS19755 (40.0 mg/kg, s.c.): vehicle + CGS19755 = 33.8 ± 5.6 vs. NMDA (40.0 mg/kg, s.c.) + CGS19755 = 13.4 ± 8.9 spontaneous tail-flicks/5 min, $P < 0.01$. In contrast, pretreatment with NMDA did not reduce the induction of spontaneous tail-flicks by dizocilpine (0.08 mg/kg, s.c.): vehicle + dizocilpine = 42.0 ± 7.5 vs. NMDA (40.0 mg/kg, s.c.) + dizocilpine = 66.3 ± 10.2 spontaneous tail-flicks/5 min. Indeed, there was a non-significant tendency towards an enhancement.

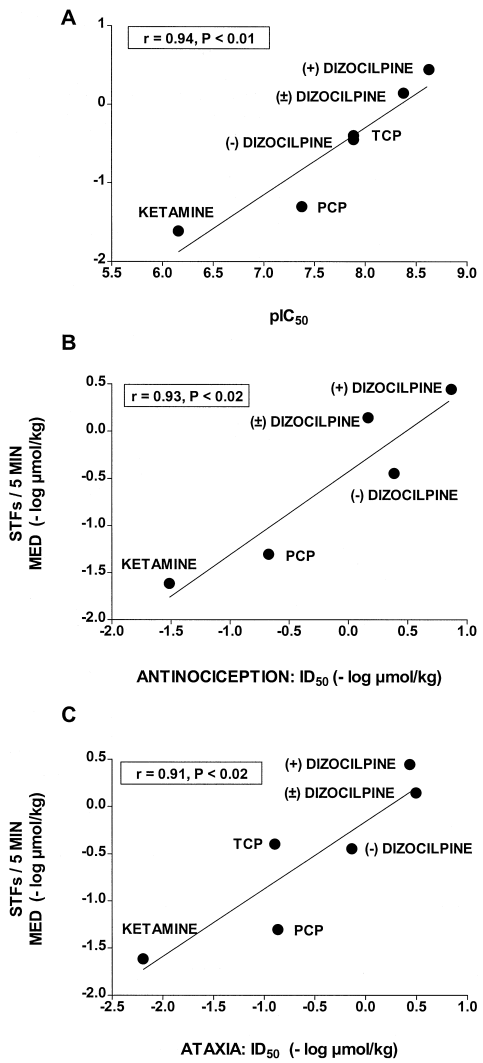

Fig. 2. Correlation analyses for open channel blockers of relationship between their potency for induction of spontaneous tail-flicks as compared to their affinity for displacement of (+)-dizocilpine binding to NMDA receptors, and their potency in inducing antinociception and ataxia. For data, see Table 1. Values are Pearson-Product Moment Correlation Coefficients.

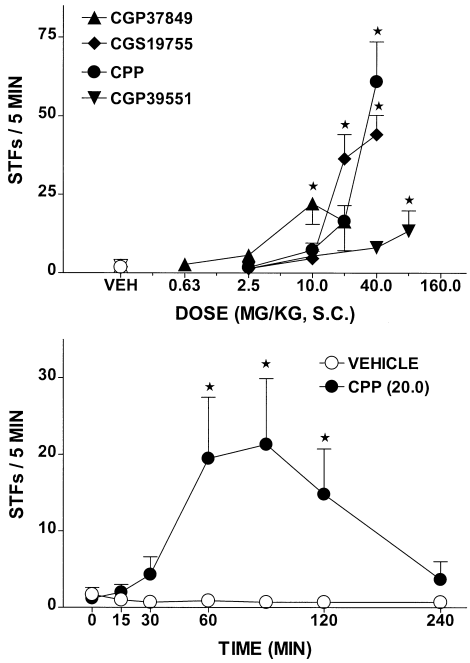

Fig. 3. Induction of spontaneous tail-flicks by systemic administration of competitive antagonists at the recognition site of the NMDA receptor. Data are means ± S.E.M. $N \geq 5$ per value. The upper panel indicates the dose–response relationship, and the lower panel shows the time-course-of-action of CPP (20.0 mg/kg, s.c.). ANOVA as follows. Upper panel: CPP, $F(4,32) = 21.7$, $P < 0.001$; CGP37849, $F(4,28) = 2.9$, $P < 0.05$; CGP39551, $F(4,31) = 2.7$, $P < 0.05$ and CGS19755, $F(4,44) = 14.8$, $P < 0.001$. Lower panel: CPP, $F(6,24) = 6.4$, $P < 0.001$ and drug × time, $F(6,36) = 3.8$, $P < 0.01$. Asterisks indicate significance of difference to corresponding vehicle values in Dunnett's test $^{*}P < 0.05$.

3.6. Induction of spontaneous tail-flicks by microinjection of dizocilpine into the nucleus accumbens

The injection of dizocilpine into the nucleus accumbens dose-dependently evoked spontaneous tail-flicks (Fig. 5). This response was pronounced and very rapid following injection. Indeed, it was apparent immediately upon termination of perfusion and placement in the recording cylinder. This effect of dizocilpine injected into the nucleus accumbens was expressed monophasically over the 16-fold range of doses examined. That is, a dose-range four-fold more extensive than the ascending component of the dose–response curve for induction of spontaneous tail-flicks by s.c. administration of dizocilpine (Fig. 1). At a dose of 5.0 μg, which elicited a full spontaneous tail-flick-response upon introduction into the nucleus accumbens, dizocilpine was ineffective in eliciting spontaneous tail-flicks upon administration into the ventral tegmental area or the striatum (Fig. 6). Further, upon intrathecal administration of 5 μg onto the dorsal surface of the lumbar spinal cord, dizocilpine likewise failed to elicit spontaneous tail-flicks (Fig. 6).

Table 2
Potency of antagonists at the NMDA receptor recognition site for induction of STFs as compared to their potencies in eliciting antinociception and ataxia
STFs expressed as MED ($P < 0.05$ to vehicle in Dunnett's test) and MOE ± S.E.M.

Drug	STFs		Antinociception[a]	Ataxia[b]
	MED	MOE ± S.E.M./ 5 min (dose)	ID_{50}	ID_{50}
CGP37849	10.0	22.6 ± 9.0 (40.0)	0.5	1.7
CGS19755	20.0	44.2 ± 6.2 (40.0)	1.7	0.8
CPP	40.0	61.0 ± 12.7 (40.0)	2.4	2.9
CGP39551	80.0	13.4 ± 6.4 (80.0)	5.9	7.8

[a]Antinociception = ID_{50} for inhibition of the late phase of licking elicited by plantar injection of formalin to mice (Millan and Seguin, 1994).

[b]Ataxia in mice = ID_{50} for reduction of latency to fall from a rotarod (Millan and Seguin, 1994).

Table 3
Lack of induction of STFs by glycine B site antagonists, as compared to their potencies in eliciting antinociception and ataxia
STFs expressed as MOE ± S.E.M. No drug actions were significant.

Drug	STFs		Antinociception[a]	Ataxia[b]
	Dose-range (*n*)	MOE ± S.E.M./ 5 min (dose)	ID_{50}	ID_{50}
L701,324	2.5–40.0 (3)	2.5 ± 0.7 (2.5)	1.5	16.5
MDL29,951	10.0–80.0 (3)	3.3 ± 2.2 (40.0)	1.0	> 80.0
ACEA1021	0.63–20.0 (3)	2.0 ± 1.4 (0.63)	5.2	9.5
L687,414	0.63–10.0 (3)	2.0 ± 1.2 (2.5)	1.9	15.5
(+)-HA966	0.63–80.0 (5)	0 ± 0 (80.0)	3.4	> 80.0

[a]Antinociception = ID_{50} for inhibition of the late phase of licking elicited by plantar injection of formalin to mice (Millan and Seguin, 1994).

[b]Ataxia in mice = ID_{50} for reduction of latency to fall from a rotarod (Millan and Seguin, 1994).

3.7. Induction of spontaneous tail-flicks by microinjection of CPP into the nucleus accumbens

In analogy to dizocilpine, the administration of CPP into the nucleus accumbens dose dependently and rapidly elicited a marked spontaneous tail-flick-response (Fig. 7).

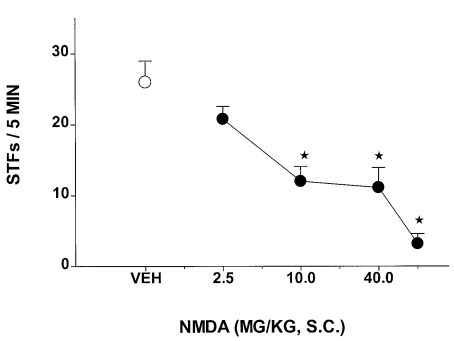

Fig. 4. Dose-dependent inhibition by NMDA of the induction of spontaneous tail-flicks by CPP. Data are means ± S.E.M. $N \geq 5$ per value. ANOVA as follows: $F(4,31) = 9.2$, $P < 0.001$. Asterisks indicate significance of difference to vehicle values in Dunnett's test $^{*}P < 0.05$.

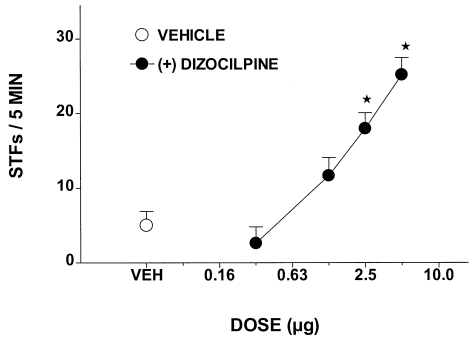

Fig. 5. Dose-dependent induction of spontaneous tail-flicks by bilateral injection of (+)-dizocilpine into the accumbens. Data are means ± S.E.M. $N \geq 5$ per value. ANOVA as follows: $F(4,18) = 17.1$, $P < 0.001$. Asterisks indicate significance of difference to vehicle values in Dunnett's test $^{*}P < 0.05$.

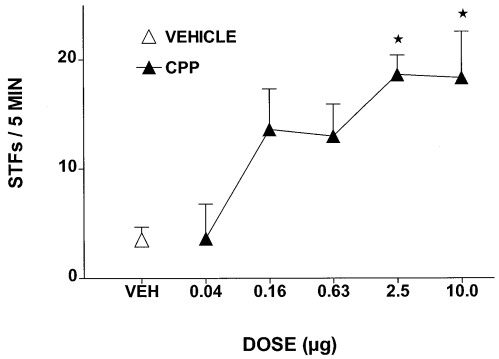

Fig. 7. Dose-dependent induction of spontaneous tail-flicks by bilateral injection of CPP into the nucleus accumbens. Data are means ± S.E.M. $N \geq 4$ per value. ANOVA as follows: $F(5,23) = 5.2$, $P < 0.05$. Asterisks indicate significance of difference to vehicle values in Dunnett's test $^{*}P < 0.05$.

The minimal effective dose of CPP for the induction of spontaneous tail-flicks upon intra-accumbens administration was ca. > 100-fold lower than that required upon s.c. application. Intrathecal administration of CPP (5–100 µg) onto the spinal cord failed to elicit spontaneous tail-flicks (not shown).

4. Discussion

4.1. Induction of spontaneous tail-flicks by blockade of NMDA receptors

Several lines of evidence support the contention that the induction of spontaneous tail-flicks by open channel blockers and recognition site antagonists reflects selective interruption of transmission at NMDA receptors. First, although phencyclidine possesses a variety of other receptorial interactions, notably at σ receptors and monoamine uptake sites (Gorelick and Balster, 1995; Millan et al., in press a), selective ligands at these receptors do not elicit spontaneous tail-flicks (Millan, 1991). Further, dizocilpine, which is virtually devoid of such interactions (Parsons et al., 1998), also elicited a robust spontaneous tail-flick-response. Second, this action of dizocilpine was expressed stereospecifically: (+)-dizocilpine was significantly more potent than its (−) counterpart, in line with its ~10-fold higher affinity at NMDA receptors (Table 1). Third, there was a pronounced correlation (Fig. 2) of open channel blocker potency for induction of spontaneous tail-flicks with affinity at NMDA binding sites and potency for induction of antinociception (and ataxia) (Millan and Seguin, 1994), prototypical effects of NMDA receptor blockade (Toru et al., 1994; Parsons et al., 1998). Further, doses of open channel blockers eliciting spontaneous tail-flicks correspond closely to those exerting antinociceptive properties (Millan and Seguin, 1994). Fourth, induction of spontaneous tail-flicks by open channel blockers was mimicked by selective antagonists at the NMDA receptor recognition site: CPP, CGS19755, CGP37849 and CGP39551 (Bennett et al., 1989; Schmutz et al., 1990; Parsons et al., 1998). The potency of these recognition site antagonists for induction of spontaneous tail-flicks also corresponded to their relative potencies for expression of their antinociceptive properties (Millan and Seguin, 1994). Fifth, NMDA attenuated the induction of spontaneous tail-flicks by the NMDA receptor recognition site antagonists, CPP and CGS19755, consistent with a competitive interaction. This action was specific inasmuch as NMDA failed to block induction of spontaneous tail-flicks by dizocilpine. As concerns the utility of the spontaneous tail-flick model for the detection of NMDA receptor antagonist properties in vivo, NMDA permits, then, differentiation of open channel blockers from NMDA receptor recognition site antagonists.

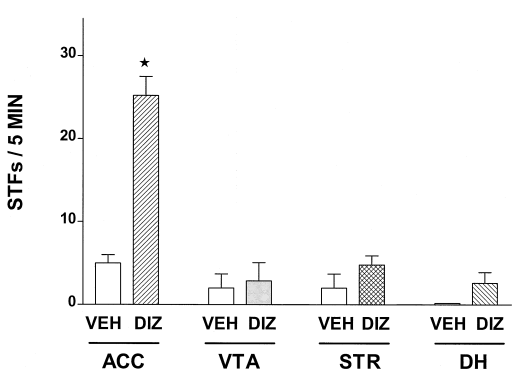

Fig. 6. Induction of spontaneous tail-flicks by administration of (+)-dizocilpine into the nucleus accumbens as compared to other central nervous system structures. Data are means ± S.E.M. $N \geq 5$ per value. The asterisk indicates significance to vehicle values in Dunnett's test (see Fig. 5). No significant differences for the other structures (Student's two-tailed *t*-test). ACC = accumbens, VTA = ventrotegmental area, STR = striatum and DH = dorsal horn of the spinal cord. Spontaneous tail-flicks were evaluated 0–5 min following administration of (+)-dizocilpine (5 µg bilaterally or 5 µg intrathecally onto the DH).

Underpinning specificity of the spontaneous tail-flick-response to NMDA receptor blockade, selective antago-

nists at AMPA/kainate receptors, such as NBQX (2,3-dihydro-6-nitro-7-sulphamoyl-benzo(f)quinoxaline) and YM90K (6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxaline dione) (Bleakman and Lodge, 1998), do not elicit spontaneous tail-flicks (Millan et al., 1996). Further, diverse drugs known to elicit hyperlocomotion, motor stereotypies and/or other stimulant effects, do not elicit spontaneous tail-flicks (see Introduction). Indeed, the only other mechanism currently known to elicit spontaneous tail-flicks is high efficacy stimulation of serotonin $(5\text{-HT})_{1A}$ receptors. However, the induction of spontaneous tail-flicks by NMDA receptor antagonists and 5-HT_{1A} receptor agonists is mediated independently by contrasting neuroanatomical substrates (Bervoets and Millan, 1994; Bervoets et al., 1993; Millan et al., 1994; Millan et al., in press b).

A key consideration in the clinical development of drugs interrupting transmission at NMDA receptors is the therapeutic window between their beneficial actions and their undesirable neurotoxic, psychotomimetic and motor-disruptive side-effects (Gorelick and Balster, 1995; Lidsky and Banerjee, 1996; Bristow et al., 1996). Although some data suggest that antagonists at the NMDA receptor recognition site possess a better security profile than open channel blockers in this regard (Tricklebank et al., 1989; Leander, 1992; Parsons et al., 1998), the underlying reasons are unclear, and differences tend to be quantitative rather than qualitative. Herein, doses required for induction of spontaneous tail-flicks by antagonists at the NMDA receptor recognition sites were (five-fold) higher than those eliciting antinociception (Millan and Seguin, 1994), whereas there was little difference in this regard for open channel blockers (Millan and Seguin, 1994). A further interesting difference between recognition site antagonists and open channel blockers as regards the induction of spontaneous tail-flicks was their monophasic vs. biphasic dose–response curves, respectively, upon s.c. administration. Interestingly, monophasic curves were seen for both CPP and dizocilpine upon their microinjection directly into the nucleus accumbens. This observation suggests that a disruptive action of open channel blockers but not NMDA recognition site antagonists at an independent population of NMDA receptors — possibly in the ventral horn — may intervene at higher doses to disrupt motor coordination and prevent expression of spontaneous tail-flicks by open channel blockers. Thus, the present data are in line with the concept that, while NMDA receptor recognition site antagonists and open channel blockers exert qualitatively similar actions, there exist subtle differences in their functional profiles.

4.2. Lack of induction of spontaneous tail-flicks by glycine B receptor antagonists

As compared to both open channel blockers and the NMDA receptor recognition site antagonists, while retaining antinociceptive, neuroprotective and anticonvulsive properties, glycine B receptor antagonists display a more benign side-effect profile in terms of a relative lack of neurotoxic actions, motor dyscoordination and psychotomimetic properties (Singh et al., 1990; Hargreaves et al., 1993; Bristow et al., 1996; Witkin et al., 1997; Parsons et al., 1998). Such an improved separation of antinociceptive vs. ataxic actions for glycine B receptor antagonists vs. open channel blockers and NMDA receptor recognition site antagonists is apparent from Table 3 which summarizes data described previously in Millan and Seguin (1994). Notably, glycine B receptor antagonists did not elicit spontaneous tail-flicks herein. In the case of (+)-HA966 and L687,414, it might be argued that their residual ($\sim$10%) efficacy at glycine B sites accounts for this difference to open channel blockers and NMDA receptor recognition site antagonists (Singh et al., 1990; Bristow et al., 1996; Priestley et al., 1996). However, endogenous pools of the full agonist, glycine, strongly activate (possibly maximally) glycine B receptors (Wood, 1995; Berger et al., 1998; Viu et al., 1998). Thus, (+)-HA966 and L687,414, by competing with glycine, clearly will reduce activity at these sites in vivo — as reflected by their antinociceptive properties. That is, their overall actions relative to that of glycine is antagonist (Millan and Seguin, 1994; Wood, 1995; Berger et al., 1998). Moreover, L701,324, MDL29,957 and ACEA1021 are pure antagonists at glycine B sites and likewise failed to elicit spontaneous tail-flicks, despite their potent antinociceptive and other properties (Bristow et al., 1996; Priestley et al., 1996) (Table 3). This question of why glycine B receptor antagonists differ to antagonists at the NMDA receptor recognition site (and open channel blockers) as regards certain functional actions is currently under study. Differences in NMDA receptor isoforms/subtypes underlying specific functional responses may account for such differences. That is, the contribution of the glycine co-agonist site at the population of NMDA receptors mediating spontaneous tail-flicks may be less pronounced than at NMDA receptors mediating nociception in the dorsal horn of the spinal cord (Dickenson, 1997; Millan, 1999). Such differences may reflect contrasting patterns of heterosubunit composition, allosteric coupling of glycine B receptors to NMDA receptor recognition sites, kinetics of channel activity and other factors (Priestley and Kemp, 1994; Grimwood et al., 1995; Porter and Greenamyre, 1995; Wood, 1995; Parsons et al., 1998; Boyce et al., 1999).

4.3. Localization of NMDA receptors underlying spontaneous tail-flicks

NMDA receptors in several CNS regions are implicated in the control of motor behaviour (Carlsson and Carlsson, 1990; Weissenborn and Winn, 1992; Ruzicka and Jhamandas, 1993; Toru et al., 1994). In fact, the bilateral introduc-

tion of dizocilpine into the nucleus accumbens elicited a dose-dependent and pronounced spontaneous tail-flick-response, whereas its introduction into the striatum or ventrotegmental area, the origin of dopaminergic projections to the nucleus accumbens, was ineffective. This suggests a high degree of neuroanatomical specificity for the induction of spontaneous tail-flicks to the blockade of nucleus accumbens-localized NMDA receptors. The rapidity of the onset of action of dizocilpine microinjections in eliciting spontaneous tail-flicks, and the relatively low doses required, strongly suggest that it exerts its actions within the nucleus accumbens. Compelling support for this contention is derived from parallel microinjection studies with CPP. In contrast to dizocilpine, CPP is a lipophobic ligand which slowly penetrates CNS tissue and which does not markedly migrate from its site of local administration — hence, its slower time-course-of-action than dizocilpine upon s.c. administration (O'Neill et al., 1989). Indeed, upon introduction into the nucleus accumbens, CPP dose-dependently and rapidly elicited a robust spontaneous tail-flick-response equivalent to that seen upon its s.c. administration, but at >100-fold lower doses.

Localization of the spontaneous tail-flick-response to the blockade of NMDA receptors in the nucleus accumbens is consistent with an extensive body of data showing that NMDA receptors in this region play an important — though complex — role in the control of motor behaviour via interactions with monoamines and other neurotransmitters (Weissenborn and Winn, 1992; Ruzicka and Jhamandas, 1993; Meltzer et al., 1997; Morari et al., 1998; Millan et al., in press a,b). Nevertheless, neuronal mechanisms underlying generation of spontaneous tail-flicks may not necessarily be related to those involved in the other locomotor actions of open channel blockers and NMDA receptor recognition site antagonists. There are two principal observations suggesting a dissociation of spontaneous tail-flicks from the induction of locomotor behaviour. First, although blockade of NMDA receptors elicits hyperlocomotion, the relevant population is probably not localized in the nucleus accumbens: indeed, activation of NMDA receptors in this region may facilitate locomotor behaviour both spontaneously and in response to the other drug classes (Hamilton et al., 1986; O'Neill et al., 1989; Pulvirenti et al., 1991; Millan et al., in press a). Second, spontaneous tail-flicks elicited by dizocilpine are potently blocked by α_2-adrenergic, but not dopamine D_2 and 5-HT_{2A} receptor, antagonists (Millan et al., in press b). In contrast, dizocilpine-elicited locomotion is resistant to α_2-antagonists, yet blocked by D_2 and/or 5-HT_{2A} antagonists (Willins et al., 1993; Martin et al., 1997; Millan et al., in press a). Further, although it might be suggested that spontaneous tail-flicks can be assimilated into the constellation of stereotyped behaviours, such as head-weaving, elicited by open channel blockers, this is questionable since such stereotypies reflect blockade of NMDA receptors in the striatum rather than the nucleus accumbens (Steinpreis, 1996). Similarly, the induction of spontaneous tail-flicks by open channel blockers and NMDA receptor recognition site antagonists can be dissociated from their ataxic effects which principally reflect blockade of NMDA receptors in the ventral horn (Toru et al., 1994; Millan, 1999) — and which are elicited independently of α_2-adrenergic receptors (Millan et al., in press b).

4.4. Summary and conclusions

To summarize, the present study shows that spontaneous tail-flicks are a novel and specific behavioural response elicited upon selective interruption of transmission at NMDA receptors by open channel blockers and NMDA receptor recognition site antagonists. Further, these two classes of antagonist may be differentiated by their relative resistance and susceptibility with respect to NMDA. In contrast, antagonists at the glycine B co-agonist site do not induce spontaneous tail-flicks, in line with studies suggesting that the functional profiles of glycine B receptor antagonists differ to those of open channel blockers and NMDA receptor recognition site antagonists. The spontaneous tail-flick-response offers, thus, a rapid and specific model for the in vivo characterization of drug actions at NMDA receptors. The population of NMDA receptors implicated in the induction of spontaneous tail-flicks is localized in the nucleus accumbens, consistent with a major role of NMDA receptors in this region in the control of motor behaviour and mood. Indeed, further examination of the functional significance of spontaneous tail-flicks would be of interest in view of the hypothesized role of deficits in glutamatergic transmission in psychotic states (Gorelick and Balster, 1995; Lidsky and Banerjee, 1996; Meltzer et al., 1997; Millan et al., in press a). In this light, spontaneous tail-flicks may provide an instructive paradigm for the exploration of functional interrelationships among NMDA receptors and monoaminergic networks as concerns the actions of antipsychotic drugs (Millan et al.,in press b).

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