Br. J. Pharmacol. (1990), **99**, 145–151

Evidence against an involvement of the haloperidol-sensitive σ recognition site in the discriminative stimulus properties of (+)-N-allylnormetazocine ((+)-SKF 10,047)

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1 The involvement of the haloperidol-sensitive, σ recognition site and the N-methyl-D-aspartic acid (NMDA) receptor in the mediation of the discriminative stimulus properties of (+)-N-allylnormetazocine ((+)-NANM, (+)-SKF 10,047), has been investigated in the rat by use of a two-lever, operant drug discrimination paradigm.

2 Six compounds with nanamolar affinity for the σ recognition site ((±)-pentazocine, (+)-3-(hydroxyphenyl)-N-propylpiperidine ((+)-3-PPP), ditolylguanidine (DTG), haloperidol, (−)-butaclamol and BMY 14802) were investigated for their ability to generalise or antagonise the (+)-NANM discriminative stimulus. Each drug was tested at doses found in an *ex vivo* radioligand binding assay to displace [^{3}H]-DTG from the central σ recognition site by more than 40%.

3 While (±)-pentazocine (in the presence of naloxone) generalised and (+)-3-PPP partially antagonised the (+)-NANM cue, the other putative σ ligands were ineffective either as agonists or antagonists at doses clearly occupying the σ site *in vivo*.

4 Dose-dependent generalisation to the (+)-NANM cue was seen with the selective non-competitive NMDA receptor antagonist, MK-801, a compound devoid of significant affinity for the σ recognition site.

5 (±)-Pentazocine was found to antagonise seizures induced in the mouse by NMDLA, a model reflecting antagonism of central NMDA receptors, and a strong correlation was found between the rank order of potency of compounds to generalise to the (+)-NANM discriminative stimulus and their potencies as anticonvulsants.

6 In conclusion, no evidence was found to substantiate the contention that the discriminative stimulus properties of (+)-NANM are mediated by the haloperidol-sensitive σ recognition site. On the other hand, the results are consistent with the interoceptive stimulus being mechanistically based in the NMDA receptor complex.

Introduction

The psychotomimetic benzomorphan drugs, typified by (+)-N-allylnormetazocine ((+)-NANM) and the arylcyclohexylamines, including phencyclidine (PCP), induce in rodents similar motor behaviours and cross-generalise in drug discrimination studies (Balster & Brady, 1982; Shearman & Herz, 1982; Brady *et al.*, 1982; Shannon, 1982; 1983). These effects were initially postulated to reflect activation of brain 'σ' receptors (Shannon, 1982; 1983; Zukin *et al.*, 1984). However, recent radioligand binding studies using the prototypical σ ligand, [^{3}H]-(+)-NANM, have demonstrated that (+)-NANM labels two distinct recognition sites in cerebral membranes: a high affinity site sensitive to nanomolar concentrations of haloperidol and a lower affinity site which corresponds to the recognition site labelled by [^{3}H]-PCP (Zukin & Zukin, 1979; Vincent *et al.*, 1979; Su, 1982; Tam, 1983; Largent *et al.*, 1984; 1986; Martin *et al.*, 1984). However, both the benzomorphans and the arylcyclohexylamines are also non-competitive antagonists of the N-methyl-D-aspartic acid (NMDA) receptor (Anis *et al.*, 1983; Berry *et al.*, 1984) and it is not clear which of these properties mediate their psychotomimetic effects.

In the present study, the involvement of the haloperidol-sensitive σ recognition site and the NMDA receptor in the generation of the discriminative stimulus induced by (+)-NANM has been investigated. Following acquisition of the discrimination, tests of stimulus generalisation and antagonism with compounds having varying degrees of selectivity for either the NMDA receptor or the sigma recognition site have been carried out. In addition, *ex vivo* radioligand binding studies with tritiated MK-801 and ditolylguanidine (DTG) as radioligands selective for the NMDA receptor channel complex and the σ recognition site, respectively (Wong *et al.*, 1986; 1988; Weber *et al.*, 1986) have been used to compare the potencies of compounds to interact with each site *in vivo* with their potencies in the drug discrimination paradigm. Potency to antagonise seizures induced in the mouse by NMDLA has also been determined as a further measure of blockade of NMDA receptor activation *in vivo*. The results provide strong evidence that the discriminative stimulus properties of (+)-NANM are mediated by an antagonist action at the NMDA receptor and argue against an involvement of the haloperidol-sensitive σ recognition site.

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Methods

Animals

Male Sprague-Dawley rats (250–300 g) and Swiss-Webster mice (18–20 g) were obtained from Bantin and Kingman, Hull, U.K. Animals were housed in groups of five under a 12 h light/dark cycle (lights on at 07 h 00 min).

Drug discrimination

Rats were deprived of food to 75–80% of their free feeding body weight and trained to discriminate the interoceptive stimulus associated with the subcutaneous administration of 3.0 mg kg^{-1} (+)-NANM from saline by a two-lever operant drug discrimination paradigm, essentially as described by

Colpaert et al. (1982) and Tricklebank et al. (1987). Briefly, 30 min before daily (Monday–Friday) training sessions, animals were injected with (+)-NANM and were required to press one of two levers (the drug lever) to obtain food reinforcement on a fixed ratio 10 (FR_{10}) schedule (one food pellet per 10 responses): on subcutaneous injection of saline, the rats were required to respond on the opposite (saline) lever. Thus, responses on the drug lever were reinforced only after injection of (+)-NANM and on the saline lever only after saline. Drug or saline administration was given according to two alternating sequences: saline, drug, drug, saline, saline or drug, saline, saline, drug, drug. The left-hand lever was designated as the drug lever in 50% of the animals and right-hand in the remainder. In any one box on any one day, left-hand and right-hand trained animals were randomised to avoid the possibility of odour cues and the lever choice of previously tested animals confounding the discrimination. Lever selection was considered correct if the animal made fewer than five responses on the inappropriate lever before completing ten responses on the appropriate one.

Generalisation or antagonism tests of five minutes duration were carried out in the absence of reinforcement once per week, commencing when the animals had achieved a correct choice in nine out of ten consecutive training sessions. In these sessions, drug lever selection was considered to have been made when the animal made fewer than ten saline lever responses before completing ten responses on the drug lever. An indication of the accuracy of the choice was obtained by noting of the error score: the number of responses on the saline lever when the number on the drug lever was ten. Animals expressing no preference for the drug lever were given the error score of ten. In both generalisation and antagonism studies drugs were given subcutaneously 30 min before the test session (i.e. putative antagonists were co-administered with (+)-NANM) except MK-801 which was administered intraperitoneally.

Seizures induced in the mouse by N-methyl-DL-aspartic acid

The ability of test compounds to protect against tonic seizures induced by N-methyl-DL-aspartic acid (NMDLA) was examined in the mouse. Animals were injected subcutaneously with NMDLA (500 mg kg^{-1}) and observed for the following 30 min. The onset of seizure activity was marked initially by wild running and barrel rolling that progressed into a tonic convulsion usually within seven minutes of injection. Putative antagonists were administered subcutaneously 15 min before injection of the convulsant. The dose of antagonist inhibiting the appearance of a seizure in 50% of the animals (ED_{50}) was calculated by probit analysis.

Ex-vivo [^3H]-*DTG and* [^3H]-*MK-801 binding*

The displacement of the specific binding of [^{3}H]-DTG and [^{3}H]-MK-801 in homogenates of whole brain following the systemic administration of σ ligands was determined in an attempt to define the minimum receptor occupancy achieved in the drug discrimination studies. Test compounds were administered subcutaneously (except MK-801 which was given intraperitoneally) 30 min before decapitation and the whole brain homogenised immediately in 11.25 volumes of ice-cold assay buffer (5 mM Tris HCl, pH 7.4). The homogenate (50 μl) was added to tubes containing 100 μl of either 20 nM [^{3}H]-DTG or 10 nM [^{3}H]-MK-801, 100 μl of buffer (to give total binding) or 100 μl of 100 μM haloperidol or thienylcyclohexylpiperidine (to define non-specific binding for the two assay systems respectively) and made up to a volume of 1 ml with assay buffer. Samples were incubated for 90 min at 25°C before rapid filtration through GF/B filters, presoaked in 0.1% polyethylenimine, followed by 2 × 5 ml washes of ice-cold buffer. Each filter was soaked in 10 ml of scintillant and the radioactivity measured by liquid scintillation spectrometry.

Drugs

The following compounds were used: (+)- and (−)-N-allylnormetazocine hydrochloride (National Institute of Drug Abuse), MK-801 ((+)-5-methyl-10,11-dihydro-5,4-dibenzo[a, d]cyclohepten-5,10-imine maleate; Merck, Sharpe and Dohme), (±)-pentazocine (Sterling Winthrop), (+)- and (−)-3-(hydroxyphenyl)-N-propylpiperidine hydrochloride (3-PPP, Research Biochemicals Incorporated), (+)- and (−)-butaclamol hydrochloride (Research Biochemicals Incorporated), naloxone hydrochloride (Sigma), N-methyl-DL-aspartic acid (Sigma), haloperidol (as the commercially available solution, Haldol), R-(−)-apomorphine hydrochloride (Research Biochemicals Incorporated) (+)-amphetamine sulphate (Sigma) and BMY 14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl) -1-piperazine butanol, Bristol-Myers). All compounds were dissolved in 0.9% NaCl except pentazocine which was dissolved in a minimum volume of 10% lactic acid and the solution neutralised with 4 M NaOH and NMDLA which was dissolved in 0.9% NaCl with the aid of 2 M NaOH and the pH adjusted to 6.5–7.0 with 2 M HCl.

Results

The (+)-NANM discriminative stimulus

Consistent and stable discrimination of 3 mg kg^{-1} (+)-NANM from saline was achieved after about 60 training sessions. Over the entire experimental period the mean error scores (±s.e.mean) for animals given (+)-NANM and saline under the conditions of the test of stimulus generalisation was 1.3 ± 0.3 (n = 116) and 9.4 ± 0.3 (n = 38), respectively. However, the dose-response relationship to (+)-NANM was extremely steep, with only 1/8 rats choosing the drug lever following a dose of 1.5 mg kg^{-1} (+)-NANM (Figure 1). In contrast, when (−)-NANM was substituted for its (+)-enantiomer, there was a clear dose-dependent selection of the (+)-NANM lever, albeit complete generalisation being achieved only at a dose some three fold greater than the training dose of (+)-NANM (Figure 1).

Compounds generalising to the (+)-NANM discriminative stimulus

Two compounds, the mixed non-competitive NMDA receptor antagonist/σ ligand, phencyclidine (0.25–2.0 mg kg^{-1}, Berry *et*

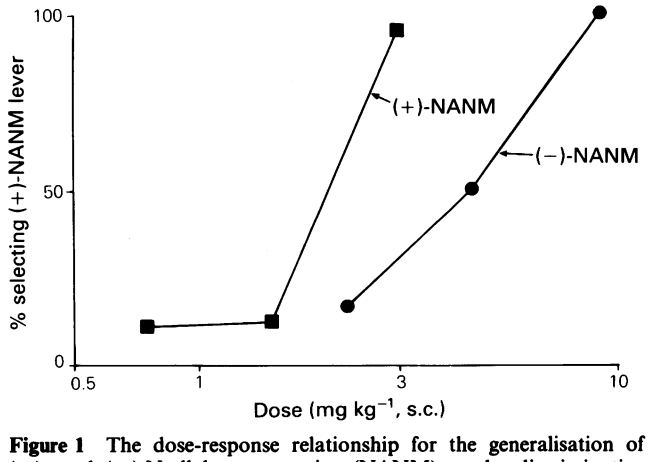

Figure 1 The dose-response relationship for the generalisation of (+)- and (−)-N-allylnormetazocine (NANM) to the discriminative stimulus induced by 3 mg kg^{-1} (+)-NANM. Values shown are the percentages of animals (n = 8 or more per dose) selecting the drug lever under the conditions of the test of stimulus generalisation (see Methods for details).

Table 1 Generalisation experiments with the (+)-N-allylnormetazocine ((+)-NANM) discriminative stimulus

Drug	*Dose* ($mg\,kg^{-1}$)	*No. responding/ No. tested*[a]	*No. choosing drug lever*[b]	*Error score*[c] (±s.e.mean)
PCP	0.25	27/29	10/27	8.0 ± 0.6
	0.5	23/23	14/23	5.1 ± 0.9
	1.0	19/23	14/19	3.3 ± 1.0
	2.0	6/8	6/6	3.2 ± 1.7
MK-801	0.075	14/14	3/14	9.2 ± 0.5
	0.15	13/14	8/13	5.1 ± 1.3
	0.3	14/20	9/14	4.4 ± 1.2
	0.5	7/14	6/7	3.4 ± 1.5
(±)-Pentazocine*	3.0	8/8	0/8	10.0
	5.0	6/8	0/8	10.0
	10.0	4/7	1/4	9.0 ± 1.0
	20.0	8/14	6/8	3.4 ± 1.5
	40.0	2/4	1/2	5.5 ± 4.5
DTG	3.0	7/7	0/7	10.0 ± 0
	10.0	8/8	1/8	9.1 ± 0.4
	30.0	6/6	1/6	9.2 ± 0.3
(−)-Butaclamol	0.1	7/7	0/7	10.0
	1.0	7/7	0/7	10.0
	4.0	7/7	0/7	10.0
(+)-Butaclamol	0.2	6/6	1/6	9.3 ± 0.7
	0.4	9/11	0/9	10.0
	0.8	9/11	0/9	10.0
	1.6	6/10	1/6	9.5 ± 0.5
(+)-3-PPP	1.0	10/12	1/10	8.7 ± 0.9
	3.0	9/12	1/9	8.3 ± 1.2
	10.0	8/11	2/8	7.9 ± 1.3
	30.0	3/10	1/3	6.7 ± 3.3
(−)-3-PPP	3.0	7/8	0/7	10.0
	10.0	5/8	0/7	10.0
	30.0	3/9	0/3	10.0
Apomorphine	0.075	5/8	0/5	10.0
	0.125	8/8	1/8	9.8 ± 0.2
	0.25	1/7	0/1	10.0
	0.5	0/5	—	—
(+)-Amphetamine	0.25	8/8	0/8	10.0
	0.5	9/9	1/9	9.3 ± 0.3
	1.0	7/9	0/7	10.0

Drugs were given subcutaneously (except MK-801 which was injected intraperitoneally) 30 min before testing. [a] The number of animals responding is the number making at least 10 responses on either the saline or (+)-NANM lever. [b] The number of animals choosing the (+)-NANM lever is the number making fewer responses on the saline lever before completing 10 on the (+)-NANM lever. [c] The error score is the mean number of responses on the saline lever when the number on the (+)-NANM lever is 10. Animals expressing no preference for the (+)-NANM lever were given a score of 10.
* (+)-Pentazocine was co-administered with naloxone ($1\,mg\,kg^{-1}$).

al., 1984), and the selective non-competitive NMDA receptor antagonist, MK-801 ($0.075–0.5\,mg\,kg^{-1}$, Wong *et al.*, 1986), dose-dependently induced (+)-NANM-appropriate responding (Table 1). Significant drug-appropriate responding was also induced by the mixed σ ligand/κ-opioid receptor agonist, (±)-pentazocine ($3.0–40.0\,mg\,kg^{-1}$, Largent *et al.*, 1984; 1986; Tam *et al.*, 1988) in the presence of the opioid receptor antagonist, naloxone ($1\,mg\,kg^{-1}$, Table 1). Naloxone alone was without significant effect. In contrast, the selective σ ligand, DTG (Weber *et al.*, 1986), and the mixed σ ligands/dopamine D_2-receptor antagonists/agonist, (+)- and (−)-butaclamol ($0.2–1.6\,mg\,kg^{-1}$ and $0.1–30\,mg\,kg^{-1}$ respectively) and (+)- and (−)-3-PPP ($1–30\,mg\,kg^{-1}$ and $3–30\,mg\,kg^{-1}$ respectively, Largent *et al.*, 1984; 1986) failed to induce significantly (+)-NANM-appropriate responding. The dopamine receptor agonist, apomorphine ($0.075–0.5\,mg\,kg^{-1}$) and the dopamine releaser, (+)-amphetamine ($0.25–1.0\,mg\,kg^{-1}$) were also without effect (Table 1).

Compounds antagonising the (+)-NANM discriminative stimulus

Five sigma receptor ligands, DTG ($3–30\,mg\,kg^{-1}$, Weber *et al.*, 1986), haloperidol ($0.025–0.4\,mg\,kg^{-1}$), (−)-butaclamol ($0.1–30\,mg\,kg^{-1}$, Su, 1982; Tam, 1983; Largent *et al.*, 1984; 1986), BMY 14802 ($1–30\,mg\,kg^{-1}$, Taylor & Dekleva, 1987) and (+)-3-PPP ($1–30\,mg\,kg^{-1}$, Largent *et al.*, 1984; 1986) were examined for their abilities to antagonise the (+)-NANM discriminative stimulus. Only the latter compound, (+)-3-PPP, significantly reduced drug-appropriate responding with 75% of the rats selecting the saline lever following a dose of $10\,mg\,kg^{-1}$ (Table 2). However, blockade of the stimulus was not further increased when the dose of (+)-3-PPP was increased to $30\,mg\,kg^{-1}$ (Table 2). Similar doses of the (−)-enantiomer of 3-PPP were without significant effect. Some attenuation of drug-appropriate responding was seen with (+)-butaclamol, but this was not clearly dose-related.

Effect of (±)-pentazocine and naloxone on seizures induced by NMDLA

(±)-Pentazocine ($20–100\,mg\,kg^{-1}$) co-administered with $1\,mg\,kg^{-1}$ naloxone dose-dependently antagonised tonic seizures induced by the subcutaneous administration of NMDLA (Table 3). Naloxone alone ($1\,mg\,kg^{-1}$) was without significant effect. NMDLA-induced seizures were also antagonised by MK-801 ($0.06–0.5\,mg\,kg^{-1}$), PCP ($2.0–10\,mg\,kg^{-1}$), (+)- and (−)-NANM ($12.5–100\,mg\,kg^{-1}$) (Table 3).

Table 2 Antagonism experiments with the (+)-N-allylnormetazocine ((+)-NANM) discriminative stimulus

Drug	Dose ($mg\,kg^{-1}$)	No. responding/ No. tested[a]	No. choosing drug lever[b]	Error score[c] (±s.e.mean)
DTG	3.0	7/8	6/7	1.7 ± 1.4
	10.0	8/8	8/8	0.4 ± 0.4
	30.0	8/8	6/8	3.7 ± 1.7
Haloperidol	0.025	7/7	7/7	0.6 ± 0.4
	0.05	6/7	5/6	3.2 ± 1.5
	0.1	4/15	4/4	2.3 ± 1.7
	0.2	4/13	3/4	2.8 ± 2.4
	0.4	1/8	1/1	0
(−)-Butaclamol	0.1	7/7	7/7	0.1 ± 0.1
	1.0	6/6	6/6	1.3 ± 1.0
	4.0	7/7	7/7	3.7 ± 1.6
	10.0	6/6	6/6	0.8 ± 0.7
	30.0	5/5	5/5	1.2 ± 1.2
(+)-Butaclamol	0.2	7/7	4/7	6.7 ± 1.5
	0.4	7/7	3/7	5.7 ± 2.0
	0.8	7/7	7/7	1.3 ± 0.8
	1.6	6/7	3/6	6.0 ± 1.7
(+)-3-PPP	1.0	7/7	6/7	3.0 ± 1.4
	3.0	7/7	4/7	6.3 ± 1.6
	10.0	4/6	1/4	8.9 ± 0.5
	30.0	16/24	5/16	7.6 ± 1.6
(−)-3-PPP	3.0	8/15	7/8	2.5 ± 1.3
	10.0	10/16	6/10	4.4 ± 1.5
	30.0	4/16	4/4	3.0 ± 2.1
BMY 14802	1.0	8/8	8/8	0
	3.0	13/15	13/13	0.8 ± 0.5
	10.0	11/14	11/11	2.0 ± 0.8
	30.0	2/10	1/2	5.0 ± 5.0

All animals received (+)-NANM ($3\,mg\,kg^{-1}$, s.c.) immediately before injection of the putative antagonists. For other details, see legend to Table 1.

Ex vivo [^3H]-*DTG and* [^3H]-*MK-801 binding*

When given 30 min before death at doses used in the drug discrimination experiments, (±)-pentazocine (10–40 $mg\,kg^{-1}$ in the presence of $1\,mg\,kg^{-1}$ naloxone), DTG (3–30 $mg\,kg^{-1}$) and (+)-3-PPP (1–30 $mg\,kg^{-1}$) dose-dependently inhibited the specific binding of [^{3}H]-DTG in whole brain homogenates (Figure 2 and Table 4). [^{3}H]-DTG binding was also inhibited dose-dependently by (−)-3-PPP, although this was approximately three fold less potent than its (+)-enantiomer (Figure 2). Haloperidol ($0.1\,mg\,kg^{-1}$), (−)-butaclamol ($30\,mg\,kg^{-1}$) and BMY 14802 ($30\,mg\,kg^{-1}$) all displaced [^{3}H]-DTG binding by more than 45% (Table 4). None of these compounds had any significant effect on [^{3}H]-MK-801 binding (results not shown).

Under identical experimental conditions, MK-801 (0.1–$1\,mg\,kg^{-1}$) has been found to dose-dependently inhibit [^{3}H]-MK-801 binding (Wong *et al.*, unpublished observations) and, in the present experiments, a dose of $1\,mg\,kg^{-1}$, which displaces 77% of specific binding, displaced only 2% of [^{3}H]-DTG binding (Table 4). In contrast, the drug discrimination

Table 3 Antagonism of N-methyl-DL-aspartic acid (NMDLA)-induced seizures in the mouse

	ED_{50} ($mg\,kg^{-1}$)	95% confidence intervals
(±)-Pentazocine*	63.3	53.4–73.6
MK-801	0.3	0.2–0.4
PCP	3.7	2.8–5.1
(+)-NANM	28.2	20.2–36.3
(−)-NANM	24.5	17.6–40.8

At least 4 doses of each antagonist were given subcutaneously (except MK-801 which was given i.p.) 30 min before subcutaneous administration of NMDLA ($500\,mg\,kg^{-1}$). Mice not exhibiting a tonic seizure within 30 min of injection of NMDLA were considered protected. The dose of antagonist protecting 50% of the animals (ED_{50}) was calculated by probit analysis. * (±)-Pentazocine was co-administered with naloxone ($1\,mg\,kg^{-1}$).

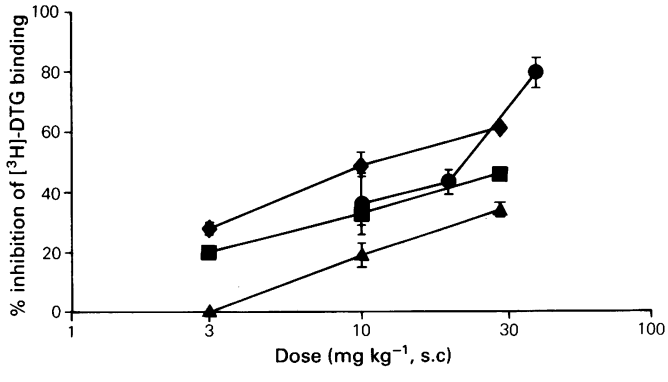

Figure 2 *Ex vivo* displacement of [^{3}H]-ditolylguanidine ([^{3}H]-DTG) binding in rat brain by σ ligands. Drugs were given subcutaneously 30 min before death. Brains were homogenised in 5 mM Tris HCl buffer, pH 7.4 and incubated with [^{3}H]-DTG as described in Methods. Specific binding was defined by 100 μM haloperidol. (◆), (+)-3-(Hydroxyphenyl)-N-propylpiperidine ((+)-3-PPP); (▲),(−)-3-PPP; (●), (±)-pentazocine; (■), DTG. Values shown are the means of the percentage displacement of binding of 6 animals per group and vertical lines indicate s.e.mean.

Table 4 *Ex vivo* displacement of [^{3}H]-ditolylguanidine ([^{3}H]-DTG) and [^{3}H]-MK-801 binding in the rat brain following subcutaneous injection of σ ligands

	Dose (mg kg^{-1})	*% displacement of binding* [^{3}H]-*DTG*	[^{3}H]-*MK-801*
(+)-NANM	3	14 ± 1	14 ± 1*
(−)-NANM	9	9 ± 1	9 ± 3*
PCP	1	20 ± 1	14 ± 2*
MK-801	1	2 ± 2	77 ± 2*
Haloperidol	0.1	46 ± 5	0
(−)-Butaclamol	30	49 ± 2	0
BMY 14802	30	57 ± 2	0
(±)-3-PPP	30	61 ± 1	0
(±)-Pentazocine	20	39 ± 5	0
	60	79 ± 5	14 ± 1*
DTG	30	46 ± 1	0

Values are the means ± s.e.mean of the percentage displacement of specific binding of [^{3}H]-DTG and [^{3}H]-MK-801 of 6 animals per group. Drugs were given subcutaneously (except MK-801 which was given i.p.) 30 min before death. Brains were homogenised in 5 mM Tris HCl buffer, pH 7.4 and incubated with [^{3}H]-DTG or [^{3}H]-MK-801 as described in Methods. Specific binding was defined by 10 μM haloperidol ([^{3}H]-DTG binding) or thienylcyclohexylpiperidine ([^{3}H]-MK-801 binding).

* $P < 0.05$ vs saline-treated controls (t test).

training dose of (+)-NANM (3 mg kg^{-1}) and doses of (−)-NANM (9 mg kg^{-1}) and phencyclidine (1 mg kg^{-1}) inducing (+)-NANM-appropriate responding gave small, but significant and quantitatively similar displacement (14–20%) of both [^{3}H]-MK-801 and [^{3}H]-DTG binding (Table 4).

Discussion

Radioligand binding studies indicate that the psychotomimetic benzomorphans and the arylcyclohexylpiperidines have appreciable affinity for at least two recognition sites in the CNS: the 'σ' recognition site sensitive to nanomolar concentrations of haloperidol and a site associated with the NMDA receptor/ion channel complex (Zukin & Zukin, 1979; Vincent *et al.*, 1979; Martin *et al.*, 1984; Su, 1982; Tam, 1983; Largent *et al.*, 1984; 1986; Wong *et al.*, 1986;1988). A number of compounds with high affinity and selectivity for one or other of these sites is now available and have been used in the present study to investigate the relative importance of the two sites in the discriminative stimulus properties of the prototypical sigma ligand, (+)-NANM. Since there is, as yet, no functional correlate of the σ recognition site, the ability of putative σ receptor agonists and antagonists to interact with the site *in vivo* has been estimated by use of an *ex vivo* radioligand binding technique. While this methodology necessarily involves dilution of the systemically administered drugs and does not, therefore, provide an accurate estimate of receptor occupancy, it does indicate whether or not a given dose of a drug is likely to have achieved a sufficiently high concentration in brain for a significant interaction with the recognition site to occur.

Effects of compounds with selectivity for the σ recognition site

Six compounds with nanomolar affinity for the σ recognition site and negligible affinity for the NMDA receptor/ion channel complex were tested for their ability to generalise to or antagonise the (+)-NANM discriminative stimulus ((±)-pentazocine, (+)-3-PPP, DTG, haloperidol, (−)-butaclamol and BMY 14802; Largent *et al.*, 1984; 1986; Weber *et al.*, 1986; Taylor & Dekleva, 1987). Each drug was tested at doses giving 45% or more displacement of [^{3}H]-DTG binding (*ex vivo*) at the time of behavioural assessment. Of these compounds, only two had an effect on the (+)-NANM discriminative stimulus. Firstly, (±)-pentazocine, in the presence of naloxone, dose-dependently induced (+)-NANM-appropriate responding, although not more than 75% of animals chose the (+)-NANM lever. This result partially contradicts the find ings of Tam *et al.* (1988) who found that in animals trained to discriminate (+)-NANM from saline, (+)-pentazocine (1.8–18 mg kg^{-1}) did not generalise, while when (+)-pentazocine was used as the training stimulus, cross-generalisation with (+)-NANM was complete. These results suggest that the interoceptive stimulus induced by (+)-NANM is a compound cue in which the 'σ' component is minor, but sufficient to indicate its presence when animals are trained on the more selective σ ligand, (+)-pentazocine.

Secondly, (+)-3-PPP was able to antagonise the (+)-NANM discriminative stimulus. Although this compound has dopamine receptor agonist properties (Hjorth *et al.*, 1981) and Steinfels *et al.* (1986) found the compound to generalise to the (+)-NANM stimulus, drug-appropriate responding was not seen in the present experiments. However, consistent with the findings of Balster (1986), (+)-3-PPP significantly antagonised the cue. The lack of effect of the (−)-enantiomer and of the dopamine receptor agonist, apomorphine, suggests that the blockade induced by (+)-3-PPP is mediated by the σ recognition site and not by dopamine receptors. The reasons for the discrepancy with the data of Steinfels *et al.* (1986) are not clear. The major difference between the studies is the use of shock avoidance rather than food reinforcement in the training procedure, as in the present work, but it is difficult to see how this might influence stimulus generalisation and/or antagonism. Despite these discrepancies, the argument for an involvement of the σ recognition site in the discriminative stimulus properties of (+)-NANM is untenable given that neither blockade nor generalisation was seen with DTG, haloperidol, (−)-butaclamol or BMY 14802 at doses that clearly occupy the σ recognition site in brain. Indeed, the complete lack of effect of DTG in the present study is significant given that low doses of the compound (3 mg kg^{-1}) have 'non-selective' discriminative stimulus properties to which not only σ ligands, but also μ and κ opioid receptor agonists generalise (Holtzman, 1989).

Role of NMDA receptors

In addition to high affinity for the σ recognition site, (+)-NANM is also a non-competitive antagonist at the NMDA receptor (Berry *et al.*, 1984). In *ex vivo* binding experiments the training dose of (+)-NANM and doses of (−)-NANM and phencyclidine inducing (+)-NANM-appropriate responding were found to displace similar amounts of binding at the NMDA receptor/ion channel complex as at the σ recognition site. The importance of this interaction with the NMDA receptor is indicated by a number of other findings. Firstly, the non-competitive NMDA receptor antagonist, MK-801, a compound devoid of significant affinity for the σ recognition site *in vitro* (Wong *et al.*, 1986; 1988) and unable to displace from the σ recognition site *ex vivo* even at the high dose of 1 mg kg^{-1}, dose-dependently generalised at modest doses to the (+)-NANM cue. (It should be noted, however, that full generalisation by MK-801 is not achieved without concomitant disruption of performance). Secondly, MK-801, (+)-NANM and 2-aminophosphonoheptanoic acid, a compound interacting competitively with the neurotransmitter recognition site on the NMDA receptor complex (Watkins & Evans, 1981; Foster & Wong, 1987) and devoid of significant affinity for the σ recognition site (Tricklebank *et al.*, 1987), induced drug-appropriate responding in animals trained to discriminate PCP from saline (Tricklebank *et al.*, 1987; Willetts

& Balster, 1988; Koek et al., 1988). Thirdly, although (±)-pentazocine has low affinity for the [^{3}H]-MK-801 recognition site (Wong et al., 1988) and does not displace [^{3}H]-MK-801 in the ex vivo binding assay, at sufficiently high doses the compound is nevertheless capable of dose-dependently antagonising seizures induced in the mouse by NMDLA, a model reflecting antagonism at NMDA receptors (Price et al., 1988; Leander et al., 1988; Tricklebank et al., 1989), at doses comparable to those generalising to the (+)-NANM cue. Indeed, relatively low doses of pentazocine given intravenously also antagonise NMDA-induced excitation in rat spinal neurones (Lodge et al., 1988).

In conclusion, the present study argues against the contention that the discriminative stimulus properties of (+)-NANM are mediated by the haloperidol-sensitive σ recognition site. On the other hand, the results are consistent with the interoceptive stimulus being mechanistically based in the NMDA receptor complex. This relationship is further illustrated by the highly significant correlation between the rank order of potency of compounds to generalise to the (+)-NANM cue and the potency to antagonise NMDLA-induced seizures (Figure 3). While a number of other behaviours induced by (+)-NANM have been proposed to reflect activation of σ receptors (e.g. Ceci et al., 1988; Walker et al., 1988), the usefulness of (+)-NANM in defining the functional correlates of the haloperidol-sensitive, σ recognition site is severely limited by its lack of selectivity. However, the present results in no way preclude the possibility that activation of the σ recognition site has important behavioural consequences.

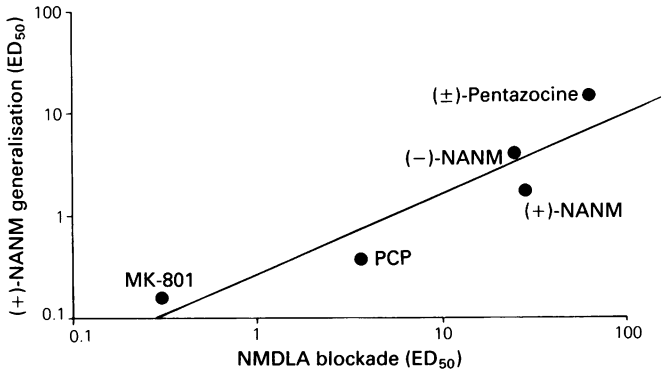

Figure 3 Correlation between the potency of compounds to block seizures induced in the mouse by N-methyl-DL-aspartic acid (NMDLA) and to generalise to the discriminative stimulus induced in the rat by (+)-N-allylnormetazocine ((+)-NANM). Data were taken from Tables 1 and 3. ED_{50} values were calculated by probit analysis.

We are grateful to Dr S. Iversen for her critical appraisal of this work and to the National Institute for Drug Abuse, Bristol-Myers and Sterling Winthrop for the gift of drugs.

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(Received June 8, 1989
Revised September 4, 1989
Accepted September 7, 1989)