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Role of the σ receptor in the inhibition of [^{3}H]-noradrenaline uptake in brain synaptosomes and adrenal chromaffin cells

Cheryl Rogers & [1]Simon Lemaire

Department of Pharmacology, Faculty of Medicine, 451, Smyth Road, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

1 Rat brain synaptosomes and cultured bovine adrenal chromaffin cells were used to monitor the inhibitory effects of phencyclidine (PCP) and sigma (σ)-receptor ligands on the uptake of [^{3}H]-noradrenaline ([^{3}H]-NA).

2 A Na^+-dependent high affinity uptake was observed in synaptosomes (30°C) and chromaffin cells (37°C) with K_m of 0.22 and 0.56 μM and V_{max} of 2.5 pmol min^{-1} mg^{-1} protein and 0.7 pmol min^{-1} per 10^6 cells, respectively.

3 PCP and haloperidol inhibited the high affinity uptake with IC_{50} of 0.17 and 0.42 μM, respectively in synaptosomes and 0.24 and 0.47 μM, respectively in adrenal chromaffin cells.

4 A close correlation ($r = 0.96$) was established between the ability of various PCP and σ-receptor ligands to inhibit [^{3}H]-NA uptake in both systems: PCP > TCP > haloperidol > 3-(+)-PPP > MK-801 $\geq$ (−)-butaclamol > (+)-SKF-10047 > DTG. Spiperone and opioid receptor ligands were ineffective at 20 μM.

5 These results indicate that the central and peripheral inhibitory effects of PCP and σ-receptor ligands on [^{3}H]-NA uptake involves a receptor (σ_1-like) which is distinct from that (PCP_2) recognized for the inhibition of [^{3}H]-dopamine uptake by PCP.

Keywords: Phencyclidine receptor; sigma receptor; catecholamine reuptake; synaptosomes; chromaffin cells; adrenal medulla

Introduction

Phencyclidine (PCP) originally introduced in 1958 as a dissociative anaesthetic has since become a major drug of abuse due to its ability to elicit hallucinations and feelings of tranquility (for review see Clouet, 1986). PCP also produces psychotic effects such as delusion, depersonalization, dysphoria and maniacal excitement that resemble the symptoms of schizophrenia (Luby *et al.*, 1959; Allen & Young, 1978). A high affinity binding site for PCP resides inside the ion channel linked to the N-methyl-D-aspartate (NMDA) receptor (Foster & Fagg, 1987). The stimulation of the PCP receptor is involved in the blockade of NMDA-evoked depolarizations of cortical and hippocampal neurones (Thompson *et al.*, 1985; Coan & Collingridge, 1987) and the inhibition of the NMDA-evoked release of dopamine and acetylcholine from brain tissue slices (Snell & Johnson, 1986; Jones *et al.*, 1987; Drejer & Honore, 1987; Snell *et al.*, 1987). PCP can also interact with a low affinity binding site, the sigma (σ)-receptor, sensitive to haloperidol and psychotic benzomorphans such as N-allylnormetazocine ((+)-SKF-10047; Su, 1982; Tam & Cook, 1984). The distinction between PCP and σ-receptors has been established by the distinct distribution of both sites in the brain (Goldman *et al.*, 1985; Largent *et al.*, 1986) and the spinal cord (Aanonsen & Seybold, 1988) and the design of selective ligands for the two receptor sites. Thus, a PCP analogue, N-[2-thienyl]cyclohexyl 3,4-piperidine, (TCP; Vignon *et al.*, 1983) and an anticonvulsant, (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d]cyclohepten-5,10-imine maleate (MK-801; Sircar *et al.*, 1987) were shown to possess a high affinity and selectivity for the PCP receptor while 3-(3-hydroxyphenyl)-N-(1-propyl)piperidine (3-(+)-PPP; Koe *et al.*, 1989) and 1,3-di(2-tolyl) guanidine (DTG; Weber *et al.*, 1986) selectively bound to the σ-receptor.

Both classes of receptors have been subdivided into two subtypes: the PCP_1 and PCP_2 receptors (Vignon *et al.*, 1986; Rothman *et al.*, 1989) and the σ_1 and σ_2-receptors (Bowen *et al.*, 1989; Hellewell & Bowen, 1990; Itzhak & Stein, 1990). Sigma (σ)-receptors are best characterized by their high affinity for benzomorphans such as (+)-SKF-10047 and their sensitivity to haloperidol. The σ_1-receptor subtype displays high affinity for 3-(+)-PPP and a stereoselective preference for (+)-benzomorphans, as well as a higher molecular weight (25–29 kDa) when compared with the σ_2-receptor subtype (18–21 kDa) (Kavanaugh *et al.*, 1989; Walker *et al.*, 1990). The σ_2-receptor demonstrates high affinity for DTG and stereospecificity for (−)-SKF-10047. All PCP and σ-receptors are insensitive to the opiate antagonist, naloxone. One of the important biological effects of PCP that may be involved in some of its psychotic properties is its blockade of catecholamine and serotonin reuptake by nerve terminals (Smith *et al.*, 1977; Bowyer *et al.*, 1984; Vignon & Lazdunski, 1984; Johnson & Snell, 1985). This effect has been ascribed to the specific interaction of the drug with the PCP_2 receptor (Snell *et al.*, 1988; Vignon *et al.*, 1988; Rothman *et al.*, 1989) as opposed to the PCP_1 receptor which is linked to the NMDA receptor complex. As yet, there has been no report to indicate if the inhibition of the uptake of catecholamines involves the stimulation of the σ receptor and if the PCP_2 receptor is identical to or distinct from the σ receptor or one of its subsets.

The adrenal medulla was recently shown to possess both PCP- and σ-like receptors (Wada *et al.*, 1988; Rogers *et al.*, 1989; Rogers & Lemaire, 1990). One site (PCP-like) was more sensitive to TCP while the other site was more sensitive to haloperidol. The putative physiological role of these binding sites has not been elucidated although PCP and the dissociative anaesthetic, ketamine, were shown to inhibit the nicotine-evoked release of catecholamines from perfused bovine adrenal glands (Malave *et al.*, 1983) and isolated adrenal chromaffin cells (Purifoy & Holz, 1984). Adrenomedullary chromaffin cells constitute a good model to study the mechanism of catecholamine reuptake (Kenigsberg & Trifaró, 1980; Role & Perlman, 1983; Banerjee *et al.*, 1987). These cells originate from the neural crest and display the same characteristics as sympathetic neurones, being able to synthesize, capture, store and release catecholamines (Stjärne, 1972). In this study, we have investigated the effect of PCP and σ-receptor ligands on the uptake of [^{3}H]-NA in bovine cultured

[1] Author for correspondence.

adrenal chromaffin cells and compared their effects with those obtained with rat isolated brain synaptosomes.

Methods

Chromaffin cell preparation

Bovine adrenal glands obtained from a local slaughterhouse were separated from their cortices and perfused *in vitro* for 10 min at 37°C with Ca^{2+}-free, Mg^{2+}-free Locke solution as described by Fenwick *et al.* (1976). Perfusion was continued for 60 min with fresh solution to which 0.05% collagenase (Sigma Chemical Co., St. Louis, U.S.A.) had been added and chromaffin cells were isolated and cultured as described previously (Fenwick *et al.*, 1976). Cells were cultured for one day and then centrifuged at 900 r.p.m. in a Beckman centrifuge for 10 min and resuspended in buffer A (composition, mM: $NaHCO_3$ 16.2, KCl 4.7, NaCl 133, $CaCl_2$ 2.5, KH_2PO_4 1.2, ascorbic acid 1.14 and nialamide 0.0124 and equilibrated at pH 7.4 with O_2:CO_2, 95%:5%) at a concentration of 5×10^6 cells ml^{-1}. Cell viability was 95% or greater by trypan blue exclusion. At this stage, cells were ready to be included in the uptake assay. The properties of [^{3}H]-NA uptake did not vary significantly from day 2 to 7 of culture (Kenigsberg & Trifaró, 1980); therefore, all assays were performed at day 2.

Preparation of rat brain synaptosomes

Male Wistar rats of the same age and weight (250–275 g) were decapitated, their brains weighed and homogenized in a glass-teflon homogenizer with a clearance of 0.025 mm, containing 0.32 M sucrose (40 ml g^{-1} wet weight). During tissue homogenization, the pestle was rotated at 800 r.p.m. with 12 up and down strokes. The homogenate was centrifuged at 1000 *g* at 4°C in a Sorvall SS34 rotor to remove nuclei and cellular debris. The resulting supernatant was centrifuged at 30,000 *g* at 4°C for 30 min. The pellets (P_2) containing crude synaptosomes were used without further purification (Smith *et al.*, 1977). The synaptosomes were resuspended in 0.32 M sucrose to yield a concentration equivalent to 1.3 mg of protein per ml (Lowry *et al.*, 1959).

Uptake studies

The uptake assay was performed by prewarming tubes containing 600 μl of buffer A, 100 μl of fixed (0.1 μM) or increasing concentrations of [^{3}H]-NA, as indicated, and 100 μl of non-labeled σ, PCP or other receptor ligands (10^{-9}–10^{-4} M) for 5 min before the addition of 200 μl aliquot of either synaptosomes (0.26 mg protein) or chromaffin cells (10^6 cells). This mixture was vortexed and incubated for 10 min (or at the indicated time) at either 0°C (control) or 30°C or 37°C as indicated. Control experiments were also performed in the absence of Na^+ by replacing NaCl and $NaHCO_3$ (buffer A) with equimolar LiCl and $LiHCO_3$, respectively. Samples were then placed on ice for 10 min before filtration by reduced pressure through Whatman GF/B filter circles (Snell *et al.*, 1988). Filters were subsequently washed with 4×3 ml of ice-cold buffer A, transferred to vials containing 8 ml of ACS scintillation cocktail (Amersham) and allowed to equilibrate overnight. Radioactivity was measured in a Beckman LS 7800 Beta counter at 37% efficiency. The total uptake at a given time point or concentration was determined by correction, subtracting the 0°C uptake (or the uptake in Na^+-free medium) from that obtained at 30°C or 37°C. The complex kinetics, composed of saturable and non-saturable components were analyzed by the method of Jaques *et al.* (1984). In the analysis of the data, the non-linear curve-fitting programme, BDATA (EMF Softwares, Knoxville, TN., U.S.A.) was used to determine the values of the K_m and V_{max} of the high affinity uptake. The concentration of PCP and σ-receptor ligands that produced 50% inhibition of [^{3}H]-NA uptake (IC_{50}) were obtained from log-logit plots. Values are means with s.e. of three separate preparations performed in duplicate.

Materials

[^{3}H]-NA (43.7 Ci $mmol^{-1}$) was obtained from New England Nuclear, Boston, MA, U.S.A. PCP and TCP were obtained from Dr H. Avdovich, Health and Welfare, Ottawa, ONT, Canada. Metaphit, (−)-butaclamol, (+)-butaclamol and 3-(+)-PPP were purchased from Research Biochemicals, Natick, MA, U.S.A. Haloperidol, nialamide, dextromethorphan and spiperone were purchased from Sigma Chemical Co., St. Louis, MO., U.S.A. BMY-14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine butanol) was obtained from Bristol Myers Co., Wallingford, CT., U.S.A. Rimcazole (BW234V: *cis*-9[3,5-dimethyl-1-piperazimyl)propyl]carbazole dihydrochloride) was donated by R.M. Ferris, Burroughs Wellcome, Research Triangle Park, NC., U.S.A. DTG was generously donated by Dr E. Weber, Portland, OR., U.S.A. (+)-SKF-10047 ((+)-N-allynormetazocine) and (−)-SKF-10047 were obtained from Natl. Inst. of Drug Abuse, Baltimore, MD., U.S.A. DAMGOL ([D-Ala^2, MePhe^4, Gly-ol^5]-enkephalin) and DSLET ([D-Ser^2, Leu^5] enkephalinyl-Thr) were supplied by Penninsula Laboratories, CA., U.S.A. U-69593 ((5α, 7α, 8β)-(+)-N-methyl-N-(7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec8yl)benzeneacetamide) was obtained from the Upjohn Co., Kalamazoo, MI., U.S.A. AP-5 (D-(−)-2-amino-7-phosphonoheptanoic acid) was purchased from Tocris Neuramin, Buckhurst Hill, Essex, U.K.

Results

Kinetics of [^{3}H]-noradrenaline uptake

Incubation of rat brain synaptosomes or bovine isolated adrenal chromaffin cells in the presence of [^{3}H]-NA (0.1 μM) at 30°C or 37°C induced a time- and temperature-dependent uptake of [^{3}H]-NA in both preparations (Figure 1). At 37°C, the synaptosomal uptake rapidly reached a maximum value (15 min) and decreased thereafter, while the chromaffin cell uptake was directly proportional to the time of incubation, up to 45 min. At 30°C, the linearity of the initial uptake lasted longer in both preparations reaching 15 min in synaptosomes and exceeding 60 min in chromaffin cells. The time of incubation was set the same (10 min) for the subsequent assays with both preparations. The linearity of the kinetics of uptake was preserved by setting the temperature at 37°C and 30°C for chromaffin cells and synaptosomes, respectively. Under these conditions, the initial rates of [^{3}H]-NA uptake were 0.65 pmol min^{-1} mg^{-1} synaptosomal protein and 0.08 pmol min^{-1} per 10^6 chromaffin cells.

Cultured adrenal chromaffin cells and isolated synaptosomes were incubated for 10 min in presence of increasing concentrations of [^{3}H]-NA at 37°C and 30°C, respectively. Both preparations displayed high and low affinity uptake processes. The high affinity uptake was Na^+-dependent and possessed the following characteristics: K_m of 0.56 and 0.22 μM for chromaffin cells and synaptosomes, respectively, and V_{max} of 0.7 pmol min^{-1} per 10^6 chromaffin cells and 2.5 pmol min^{-1} mg^{-1} synaptosomal protein. The low affinity uptake was not characterized. Incubation in the absence of Na^+ resulted in complete abolition of the high affinity uptake process without affecting the low affinity uptake. All experiments were then performed at a concentration (0.1 μM) at which [^{3}H]-NA uptake occurred through the Na^+-dependent high affinity site.

Inhibition of [^{3}H]-noradrenaline uptake by PCP and sigma-receptor ligands

The uptake of [^{3}H]-NA was examined in the presence of increasing concentrations of PCP and σ-receptor ligands (Figure 2, Table 1). PCP and haloperidol caused a dose-

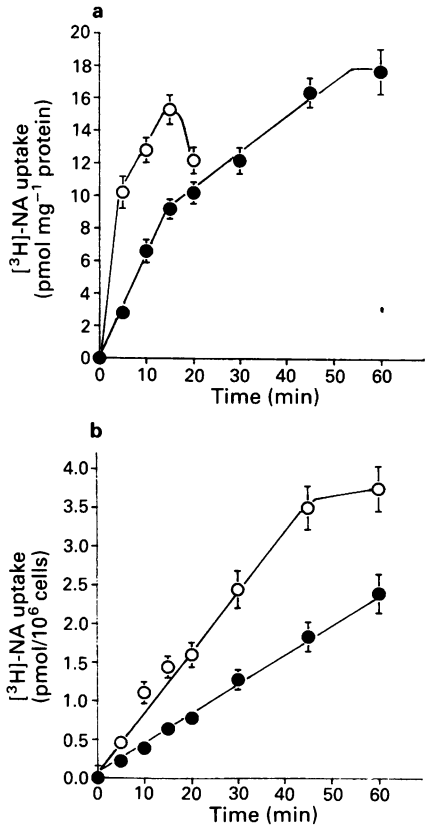

Figure 1 Time- and temperature-dependence of apparent [^{3}H]-noradrenaline ([^{3}H]-NA) uptake into rat brain synaptosomes (a) and bovine cultured adrenal chromaffin cells (b). Synaptosomes and chromaffin cells were incubated at 30°C (●) or 37°C (○) in the presence of [^{3}H]-NA (0.1 μM). Uptake was terminated at various times as described in Methods. Each point represents mean uptake of three sets of duplicates; s.e. shown by vertical bars.

dependent inhibition of [^{3}H]-NA uptake with total inhibition occurring at a concentration of 10–100 μM. The concentration of PCP or haloperidol producing 50% inhibition was quite similar in both systems for PCP (0.17 and 0.24 μM) and for haloperidol (0.42 and 0.47 μM). In order to characterize better the receptor involved in the inhibition of catecholamine uptake, the inhibitory potencies of various specific ligands for PCP and σ-receptors were compared in both uptake systems (Table 1). Among the various PCP receptor ligands tested, PCP and TCP were the most potent (IC_{50} of 0.17–0.42 μM) while MK-801, a specific ligand for the PCP_1 receptor, was less active (IC_{50}: 1.92 and 4.90 μM in chromaffin cells and synaptosomes respectively). The σ-receptor ligands, haloperidol, 3-(+)-PPP, dextromethorphan and rimcazole displayed intermediate potency between PCP and MK-801 with IC_{50} ranging between 0.42 and 4.03 μM. However, other selective σ-receptor ligands such as (+)-SKF-10047, BMY-14802 and DTG were less potent (IC_{50} range of 7.24 to 29.7 μM). The σ-receptor ligand, (+)-SKF-10047, displayed stereoselectivity for the inhibition of [^{3}H]-NA uptake, the (+)-isomer being approximately 3 times more potent than the (−)-isomer in both systems. However, the stereoselective preference for (−)-butaclamol was less evident (Table 1). The D_2 receptor ligand, spiperone, the selective opioid agonists, DAMGOL (μ), DSLET (δ) and U-69593 (κ) and the NMDA receptor antagonist, AP-5 were ineffective at 20 μM.

Table 1 Inhibition of [^{3}H]-noradrenaline ([^{3}H]-NA) uptake by phencyclidine (PCP) and σ-receptor ligands in rat brain synaptosomes and bovine cultured adrenal chromaffin cells

Drug	*Synaptosomes* IC_{50} (μM)[a]	*Chromaffin cells* IC_{50} (μM)[a]
Desmethylimipramine	0.03 ± 0.009	0.01 ± 0.002
PCP ligands		
PCP	0.17 ± 0.09	0.24 ± 0.07
TCP	0.34 ± 0.08	0.42 ± 0.08
Metaphit	0.75 ± 0.11	0.68 ± 0.12
MK-801	4.90 ± 1.34	1.92 ± 0.18
Sigma ligands		
Haloperidol	0.42 ± 0.11	0.47 ± 0.09
3-(+)-PPP	0.82 ± 0.07	0.50 ± 0.08
Dextromethorphan	1.73 ± 0.31	0.51 ± 0.02
Rimcazole	1.96 ± 0.78	4.03 ± 0.28
(−)-Butaclamol	4.06 ± 0.89	5.11 ± 0.12
(+)-Butaclamol	6.06 ± 0.97	8.25 ± 0.15
(+)-SKF-10047	7.24 ± 1.45	14.0 ± 2.87
BMY-14802	9.45 ± 1.16	10.5 ± 2.45
(−)-SKF-10047	20.5 ± 2.46	38.3 ± 6.04
DTG	29.7 ± 2.37	20.0 ± 4.24

[a] The IC_{50} of spiperone (D_2), DAMGOL (μ), DSLET (δ), U-69593 (κ), and AP-5 (NMDA) were ineffective at 20 μM in both systems.
For abbreviations, see text.

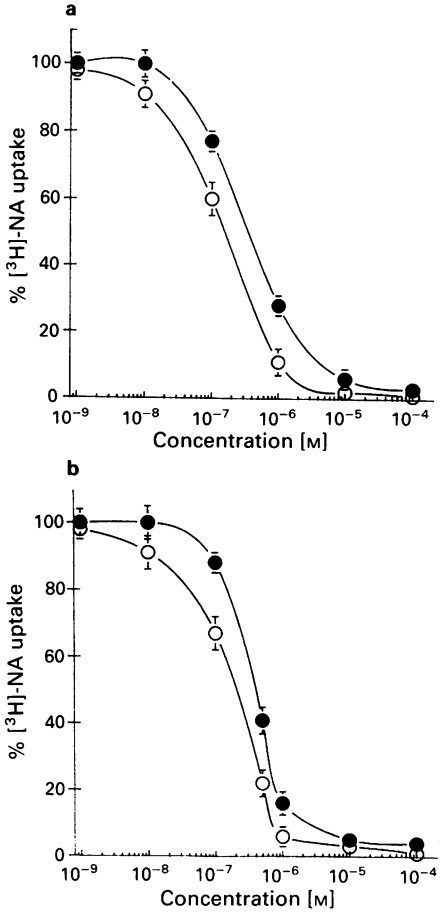

Figure 2 Effects of phencyclidine (PCP) (○) and haloperidol (●) on the uptake of [^{3}H]-noradrenaline ([^{3}H]-NA) into rat brain synaptosomes (a) and bovine cultured adrenal chromaffin cells (b). Synaptosomes and chromaffin cells were incubated at 30°C and 37°C respectively, for 10 min in presence of [^{3}H]-NA (0.1 μM) and increasing concentrations of PCP or haloperidol. Uptake was terminated as described in Methods. Each point represents mean uptake of three sets of duplicates; s.e. shown by vertical bars.

Discussion

The present study indicates that both σ- and PCP-receptor ligands inhibit the uptake of [^{3}H]-NA in rat brain synaptosomes and bovine cultured adrenal chromaffin cells. A

close correlation ($r = 0.96$) exists between the order of potency of these drugs in inhibiting the uptake of [^{3}H]-NA in the two systems (Figure 3), suggesting that the mechanism that mediates this effect is probably the same in the brain and peripheral tissues. The uptake systems for [^{3}H]-NA in crude synaptosomal preparations (for a review see Iversen, 1975) and isolated adrenal chromaffin cells (Kenigsberg & Trifaró, 1980; Role & Perlman, 1983; Jaques *et al.*, 1984; Banerjee *et al.*, 1987) have been described. A high affinity uptake system exists in both tissues. This uptake is temperature- and Na^+-dependent, sensitive to ouabain, cocaine and desmethylimipramine and is saturable and stereospecific. A temperature-sensitive low affinity uptake was also described as Na^+-independent, cocaine-insensitive, non-saturable and not stereospecific. A physiological role for the low affinity uptake site in normal neurotransmission is less probable, although it appears to play an important role in the pharmacological responses to sympathomimetics (Hoffman & Lefkowitz, 1990). Interestingly, the inhibitory effects of PCP and σ-receptor ligands were related to the high affinity uptake (Table 1).

PCP and σ-receptor ligands have multiple binding sites in membrane preparations of rat brain (Quirion *et al.*, 1987; Bowen *et al.*, 1989; Haring *et al.*, 1990; Itzhak & Stein, 1990). The high affinity binding site for PCP is located inside the NMDA receptor-linked ion channel (Foster & Fagg, 1987). A lower affinity PCP binding site was also characterized by use of the prototypic drug, N-[1-(2-benzo (b) thiophenyl) cyclohexyl] piperidine (BTCP), an analogue of PCP, the binding of which was Na^+-dependent. There was also a good correlation between the affinity of compounds to displace the binding of [^{3}H]-BTCP from the PCP_2 site and their potency to inhibit [^{3}H]-dopamine uptake (Vignon *et al.*, 1988). The σ receptor is less well defined but, in the brain, its localization is distinct from that of the PCP_1 binding site (Largent *et al.*, 1986) and it can also be subdivided into two receptor subtypes (Bowen *et al.*, 1989; Hellewell & Bowen, 1990; Itzhak & Stein, 1990).

PCP and σ-like receptors in membrane preparations of bovine adrenal medulla have also been observed (Rogers *et al.*, 1989; Rogers & Lemaire, 1990). It appears that the PCP_1 receptor (NMDA linked) for which MK-801 possesses nanomolar affinity is not present in the adrenal medulla. The adrenomedullary [^{3}H]-TCP binding is sensitive to haloperidol and its pharmacological profile is distinct from both PCP_1 and PCP_2 receptors but rather resembles that of the σ_1-receptor (Rogers & Lemaire, 1990). Yet, the order of potency of PCP and σ-receptor ligands in the inhibition of [^{3}H]-NA uptake in brain synaptosomes and isolated adrenal chromaffin cells is similar ($r = 0.96$; Figure 3), indicating that the receptor(s) involved must possess the same characteristics. This order of potency (PCP > TCP > 3-(+)-PPP > MK-801 > (+)-SKF-10047) also corresponds to the rank order of potency for the potentiation of the contractile response of NA in the rat tail artery (TCP > 3-(+)-PPP > MK-801(+)-SKF-10047) (Massamiri & Piper Duckles, 1989), but not to the order of potency observed for the inhibition of [^{3}H]-BTCP binding to the PCP_2 site, and the blockade of [^{3}H]-dopamine uptake in striatal synaptosomes (Vignon *et al.*, 1988). Since the adrenal medulla contains no PCP_1 binding site, it may prove to be a useful tissue in which to examine the nature of the receptor(s) involved in the inhibition of [^{3}H]-NA uptake by PCP and σ-receptor ligands.

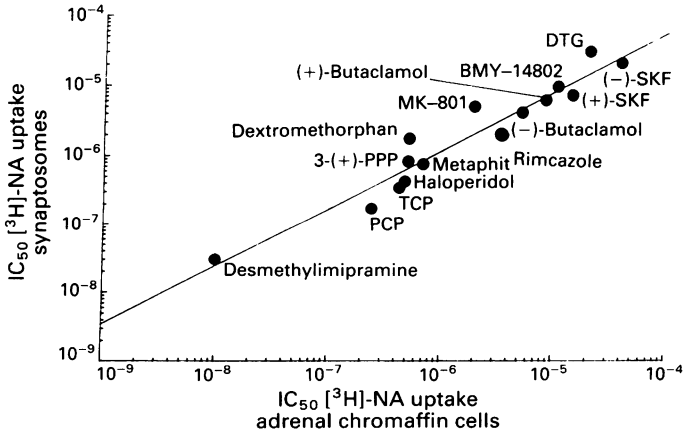

Figure 3 Correlation between the potencies of various phencyclidine (PCP) and σ-receptor ligands in inhibiting the uptake of [^{3}H]-noradrenaline ([^{3}H]-NA) in rat brain synaptosomes and bovine cultured chromaffin cells. The values were taken from Table 1 ($r = 0.96$; (+)- or (−)-SKF = (+)- or (−)-SKF-10047). For other abbreviations, see text.

The brain PCP_2 receptor, responsible for the inhibition of [^{3}H]-dopamine uptake, has been well characterized by the use of the PCP analogue, BTCP, which selectively binds to this receptor and potently inhibits the uptake of [^{3}H]-dopamine (IC_{50}: 13.9 nM; Vignon *et al.*, 1988). However, BTCP appeared to be a relatively poor inhibitor of [^{3}H]-NA uptake (IC_{50} > 10 μM) indicating that this latter effect of PCP-like compounds may involve a distinct receptor. The possible involvement of a distinct receptor was also supported by the relative high potency of MK-801 in the inhibition of [^{3}H]-NA uptake as compared with its inefficiency in inhibiting [^{3}H]-dopamine uptake (Snell & Johnson, 1988; Table 1). In addition, the drug TCP, a potent inhibitor of [^{3}H]-NA uptake in both rat brain synaptosomes and bovine adrenal chromaffin cells (Table 1), was a poor inhibitor of [^{3}H]-BTCP binding and [^{3}H]-dopamine uptake (Vignon *et al.*, 1988) and the displacement of [^{3}H]-TCP binding required high concentrations of BTCP (Rothman *et al.*, 1989).

In the adrenal medulla, [^{3}H]-TCP displayed one high affinity binding component that was potently displaced by haloperidol (K_i: 19 nM) and stereoselectively inhibited by (+)-SKF-10047 (Rogers & Lemaire, 1990), a characteristic of the σ_1-receptor subtype (Walker *et al.*, 1990). Interestingly, the σ-receptor ligands, haloperidol and 3-(+)-PPP were also potent inhibitors of [^{3}H]-NA uptake in rat brain synaptosomes and adrenal chromaffin cells (Table 1). DTG has been shown to be a potent ligand for both σ_1- and σ_2-receptors (Walker *et al.*, 1990), but the effects of GTP on the competition of [^{3}H]-3-(+)-PPP binding by DTG was negligible (Beart *et al.*, 1989), indicating that it may be acting as an antagonist on the 3-(+)-PPP binding site (σ_1; Walker *et al.*, 1990). We did not find any antagonistic activity for DTG in the [^{3}H]-NA uptake assays against PCP or 3-(+)-PPP (data not shown), however, its weak agonist activity (Table 1) may reflect that it is not acting as a pure agonist.

The stereoselective inhibition of [^{3}H]-NA uptake by (+)-SKF-10047 rather than by (−)-SKF-10047 indicates that the receptor involved may be the σ_1 subtype (Hellewell & Bowen, 1990). On the other hand, the similarity between the molecular weights of the σ_1-receptor (25–29 kDa; Kavanaugh *et al.*, 1989; Walker *et al.*, 1990) and that of the PCP_2 receptor (33 kDa; Haring *et al.*, 1987) but not that of the σ_2 (18–21 kDa; Hellewell & Bowen, 1990) or the PCP_1 receptor (90 kDa; Haring *et al.*, 1987) may indicate that the receptors involved in the blockade of dopamine uptake (PCP_2) and NA uptake (σ_1) have a structure that is quite similar. Finally, the NA carrier on which PCP exerts a competitive action (Smith *et al.*, 1977; Schömig *et al.*, 1988) also appears to have a molecular weight (69 kDa; Pacholczyk *et al.*, 1991) that is distinct from those of the PCP_2 and σ_1-receptors.

A clear distinction between all those receptor subtypes will necessitate their isolation and complete structural identification but our results indicate that the selective inhibition of [^{3}H]-NA uptake by PCP and σ-like compounds in the brain and periphery probably involves a receptor site that is distinct from that for the inhibition of [^{3}H]-dopamine uptake (PCP_2 receptor) and which possesses similarities with the σ_1-receptor subtype.

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