Br. J. Pharmacol. (1994), **112**, 709–715

The effects of sigma ligands and of neuropeptide Y on N-methyl-D-aspartate-induced neuronal activation of CA_3 dorsal hippocampus neurones are differentially affected by pertussin toxin

François P. Monnet, [1]Guy Debonnel, Richard Bergeron, Benjamin Gronier & Claude de Montigny

Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University, 1033 Pine Avenue, West, Montréal, Québec, Canada H3A 1A1

1 The *in vivo* effects of the high affinity sigma ligands 1,3-di(2-tolyl)guanidine (DTG), (+)-N-cyclopropylmethyl-*N*-methyl-1,4-diphenyl-1-ethyl-but-3-en-1-ylamine hydrochloride (JO-1784), (+)-pentazocine and haloperidol, as well as of those of neuropeptide Y (NPY), on *N*-methyl-D-aspartate (NMDA)- and quisqualate (Quis)-induced neuronal activations of CA_3 pyramidal neurones were assessed, using extracellular unitary recording, in control rats and in rats pretreated with a local injection of pertussis toxin (PTX), to evaluate the possible involvement of $G_{i/o}$ proteins in mediating the potentiation of the neuronal response to NMDA by the activation of sigma receptors in the dorsal hippocampus.

2 Microiontophoretic applications as well as intravenous injections of (+)-pentazocine potentiated selectively the NMDA response in control rats as well as in PTX-pretreated animals. In contrast, the PTX pretreatment abolished the potentiation of the NMDA response by DTG, JO-1784 and NPY. Moreover, microiontophoretic applications of DTG induced a reduction of NMDA-induced neuronal activation. Neither in control nor in PTX-treated rats, did the sigma ligands and NPY have any effect on Quis-induced neuronal response.

3 In PTX-treated rats, the potentiation of the NMDA response induced by (+)-pentazocine was suppressed by haloperidol, whereas the reduction of the NMDA response by DTG was not affected by haloperidol.

4 This study provides the first *in vivo* functional evidence that sigma ligands and NPY modulate the NMDA response by acting on distinct receptors, differentiated by their PTX sensitivity.

Keywords: NMDA; $G_{i/o}$ protein; pertussis toxin; sigma receptors; haloperidol; 1,3-di(2-tolyl)guanidine (DTG); JO-1784; (+)-pentazocine; neuropeptide Y; BMY-14802

Introduction

The recent synthesis of selective and high affinity sigma ligands has led to the identification of some of the pharmacological characteristics of sigma receptors. We have shown that several sigma ligands selectively and markedly potentiate *N*-methyl-D-aspartate (NMDA)-induced neuronal activation of rat CA_3 dorsal hippocampus pyramidal neurones using an *in vivo* electrophysiological paradigm (Monnet *et al.*, 1990; 1992b). This potentiation of the NMDA response is suppressed by other sigma ligands such as haloperidol, BMY-14802 and (+)-*N*-n-propyl-3-(3-hydroxyphenyl) piperidine [(+)-3-PPP] which by themselves, at low doses, do not affect the NMDA response. Therefore, the former are tentatively denoted agonists and the later antagonists in the present manuscript.

These results suggest the existence of a functional interaction between sigma and NMDA receptors. Further support for this notion has been provided by data obtained in both A9 and A10 regions (Iyengar *et al.*, 1990a) and in the hypothalamic-pituitary-adrenal axis (Iyengar *et al.*, 1990b; 1991). Neuropeptide Y (NPY), which has been reported to bind to sigma receptors (Roman *et al.*, 1989; 1993; Bouchard *et al.*, 1993), behaves like sigma agonists in our *in vivo* electrophysiological model (Monnet *et al.*, 1992c,d) as well as in various *in vivo* and *in vitro* paradigms (Roman *et al.*, 1989; 1991b; Riviere *et al.*, 1990), suggesting that it might act on sigma receptors.

Guanosine triphosphate (GTP)-binding regulatory G proteins (guanine nucleotide-binding proteins) might be involved in the biological effects of several high affinity sigma ligands (Itzhak & Khouri, 1988; Chattarji *et al.*, 1989; Itzhak, 1989). Indeed, in rat brain membrane preparations, GTP and Gpp(NH)p reduce the high affinity component of the binding of the sigma ligands (+)-*N*-allylnormetazocine [(+)-SKF-10,047], (+)-3-PPP and pentazocine, this phenomenon being attributed to the conversion of sigma receptors from a high to a low affinity state (Irzhak & Khouri, 1988; Beart *et al.*, 1989). As Gpp(NH)p reduces the slower dissociative component of sigma binding, it has been proposed that, in their high affinity state, at least a subpopulation of sigma receptors are coupled to G proteins (Itzhak, 1989). In addition, pretreatment with pertussis toxin (PTX), which inactivates $G_{i/o}$ proteins by ADP-ribosylation, reduces the high affinity binding component and prevents the effects of Gpp(NH)p on [^{3}H]-(+)-3PPP binding (Itzhak, 1989), suggesting the coupling of sigma receptors to $G_{i/o}$ proteins (Gilman, 1987; Fredholm & Lindgren, 1988; Hertting & Allgaier, 1988).

Sigma receptors have been separated, to date, into two classes, denoted σ_1 and σ_2 (Quirion *et al.*, 1992). The sigma ligands, haloperidol, 1,3-di(2-toly)guanidine (DTG) and (+)-3-PPP, do not discriminate between σ_1 and σ_2 sites, whereas the (+)-benzomorphans, (+)-SKF-10,047 and (+)-pentazocine as well as JO-1784, bind preferentially to σ_1 sites with a nanomolar affinity (Quirion *et al.*, 1992).

[1] Author for correspondence.

The goal of the present study was thus to assess, *in vivo*, whether the effect of sigma ligands on the NMDA response involved a $G_{i/o}$ type of protein. To this end, using *in vivo* extracellular unitary recording of CA_3 dorsal hippocampus pyramidal neurones, we compared in control rats and in rats pretreated with PTX, the capacity of high affinity sigma ligands (DTG, JO-1784, (+)-pentazocine) and of NPY, to potentiate NMDA-induced neuronal activation in the CA_3 dorsal hippocampus.

Methods

Pertussis toxin pretreatment

Male Sprague-Dawley rats (175–200 g) were obtained from Charles River (Saint-Constant, Québec, Canada) and housed four per cage. They were kept on a 12 h:12 h light/dark cycle with free access to water and Purina chow. Following anaesthesia with chloral hydrate (400 mg kg^{-1}, i.p.), the rats were mounted in a stereotaxic apparatus. The pretreatment with PTX (1 μg in 2 μl of physiological saline, Sigma Chemical Co., St-Louis, MO, U.S.A.) consisted in lowering unilaterally the tip of a 5-μl Hamilton syringe into the dorsal hippocampus at A: 4.5, L: 4 and D: 4, according to the atlas of Paxinos & Watson (1986). Pertussis toxin was slowly injected over a period of 5 min. Control rats received an equal volume of physiological salines. *In vivo* electrophysiological experiments were carried out 3 to 11 days later. This interval was chosen because it was observed, in the same *in vivo* electrophysiological experiments, that the efficacy of microiontophoretic applications of 5-hydroxytryptamine (5-HT) in suppressing the firing activity of CA_3 hippocampal pyramidal neurones, an effect which is $G_{i/o}$ protein-dependent (Andrade *et al.*, 1986), was drastically reduced during this time period (Blier *et al.*, 1992).

Recordings from CA_3 dorsal hippocampus pyramidal neurones

Recordings were obtained as previously described (Monnet *et al.*, 1992d). In brief, rats were anaesthetized with urethane (1.25 g kg^{-1}, i.p.), and mounted in a stereotaxic apparatus. Body temperature was maintained at 37°C throughout the experiment. Five-barrelled glass micropipettes, were used for extracellular unitary recordings of the activity of CA_3 dorsal hippocampus pyramidal neurones. One side barrel, filled with 2 M NaCl, was used for automatic current balancing. The other side barrels, used for microiontophoresis, were filled with NMDA (10 mM in 200 mM NaCl, pH 8), Quis (1.5 mM in 400 mM NaCl, pH 8), acetylcholine (ACh) (1.5 mM in 200 mM NaCl, pH 8) and one of the following solutions: DTG (1 mM in 200 mM NaCl, pH 4), JO-1784 (1 mM in 200 mM NaCl, pH 4), (+)-pentazocine (0.5 mM in 200 mM NaCl, pH 4), NPY (0.1 mM in 150 mM NaCl and bovine serum albumin 0.1%, pH 4), or haloperidol (0.2 mM in 50 mM NaCl, pH 3.5).

The micropipette was lowered into the CA_3 region of the dorsal hippocampus (L: 4.2 mm and A: 4.2 mm, at a depth of 3.5 to 4.5 mm from the cortical surface; Paxinos & Watson, 1986). Action potentials, monitored on an oscilloscope, triggered square pulses fed to a computer. The duration of the microiontophoretic applications and the intensities of the currents used were also stored in the computer, permitting the calculation of the total number of spikes generated per nC. For a given neurone, the currents of NMDA and Quis were adjusted to obtain a firing frequency in between 7 and 15 Hz and were thereafter maintained constant for the reminder of the experiment. All applications of NMDA and Quis were of 50 s, while those of the sigma ligands and of NPY were of 15 to 20 min.

Experimental series

In a first series of experiments, since NPY does not cross the blood brain barrier and could only be applied by microiontophoresis, the sigma ligands DTG, JO-1784, (+)-pentazocine and haloperidol were also applied by microiontophoresis in control and PTX-treated animals. The effects of the microiontophoretic applications of the sigma ligands were assessed by determining the number of spikes generated per nC of NMDA and Quis before and during the microiontophoretic application of the substance studied.

In a second series, DTG, (+)-pentazocine and haloperidol were administered intravenously. The effects of these sigma ligands were assessed by determining the number of spikes generated per nC of NMDA and Quis before and after the injection. Only one neurone was tested in each rat which received only one dose of DTG or (+)-pentazocine.

Drugs

The following substances were used: NMDA and PTX (Sigma Chemical, St. Louis, MO, U.S.A.), Quis (Tocris Neuramin, Buckhurst Hill, Essex, U.K.), DTG (Aldrich, Milwaukee, WI, U.S.A.), haloperidol (McNeil Laboratories, Stouffville, ONT, Canada). JO-1784 [(+)-N-cyclopropylmethyl-*N*-methyl-1,4-diphenyl-1-ethyl-but-3-en-1-ylamine, hydrochloride] was kindly provided by Dr J.L. Junien (Institut de Recherche Jouveinal, Fresnes, France), (+)-pentazocine by Dr B.C. deCosta (N.I.H., Bethesda, MD, U.S.A.) and NPY was a generous gift from Dr A. Fournier (Institut National de la Recherche Scientifique-Santé, Pointe-Claire, Québec, Canada).

Statistical analysis

All results are expressed as the mean ± s.e.mean of the number of spikes generated per nC of NMDA or Quis, *n* being the number of neurones tested. Statistical significance was assessed by Student's paired *t* test with Dunnett's correction for multiple comparisons. Probability values less than 0.05 were considered as significant.

Results

Microiontophoretic applications of NMDA and Quis produced consistent activation of all CA_3 pyramidal neurones recorded. Pertussis toxin, which inactivates $G_{i/o}$ proteins by ADP-ribosylation, was used to document the possible involvement of these proteins in the modulation of NMDA-induced neuronal activation in the CA_3 region of the rat dorsal hippocampus by high affinity sigma ligands and NPY. The *in vivo* PTX pretreatment did not affect the spontaneous firing activity or NMDA- or Quis-induced neuronal activity. None of the sigma ligands tested nor NPY had any effect on the spontaneous activity of pyramidal neurones in the CA_3 region, consistent with previous observations (Brooks *et al.*, 1987; Lodge *et al.*, 1988; Monnet *et al.*, 1992b,c,d).

Effects of microiontophoretic applications of JO-1784, NPY, DTG and (+)-pentazocine

In a first series of experiments, the high affinity sigma ligands JO-1784 (Roman *et al.*, 1990), (+)-pentazocine (Su, 1982), DTG (Weber *et al.*, 1986), as well as NPY (Roman *et al.*, 1989) were applied microiontophoretically for successive periods of 10 to 20 min with 5, 10 and 20 nA ejecting currents, in control and PTX-treated rats.

In naive rats, JO-1784 (from 10 and 20 nA) produced a selective and current-dependent enhancement of the NMDA response, 20 nA inducing a three fold increase of NMDA-induced firing activity (Figures 1a, 2a). In PTX-treated rats, JO-1784, even at a current of 20 nA, failed to induce any

significant potentiation of the NMDA response (Figures 1b, 2a). As in control rats, JO-1784 did not modify the neuronal response to Quis.

Similarly, when applied microiontophoretically with a current of 10 nA, NPY produced in control rats a two fold enhancement of the NMDA response (Figure 2b), but had no effect on the Quis response. In PTX-treated rats, the potentiating effect of NPY on the NMDA response was abolished (Figure 2b).

In control animals, microiontophoretic applications of DTG produced a greater than two fold increase in the neuronal activation induced by microiontophoretic applications of NMDA, (Figure 2c). After pretreatment with PTX however, DTG induced a slight but significant reduction of the NMDA-induced firing activity (Figure 2c). The Quis response was not modified by the microiontophoretic application of DTG in either control or PTX-pretreated rats.

The microiontophoretic application of (+)-pentazocine produced, in naive rats, a 150% increase of NMDA-induced neuronal activation (Figure 2d), without affecting the response to Quis. In PTX-treated rats, (+)-pentazocine, unlike the other sigma ligands, still produced a potentiation of the NMDA response (Figure 2d), without modifying that of Quis.

As in previous studies, in control animals, the microiontophoretic application, as well as the intravenous administration of a low dose (10 $\mu g\,kg^{-1}$), of haloperidol concurrently with the application of DTG, (+)-pentazocine, JO-1784 or NPY, suppressed the potentiation of the NMDA response induced by these sigma ligands (data not shown). In PTX-treated rats, the potentiation induced by the microiontophoretic application of (+)-pentazocine was also suppressed by haloperidol (10 $\mu g\,kg^{-1}$, i.v., Figure 2d). However, the

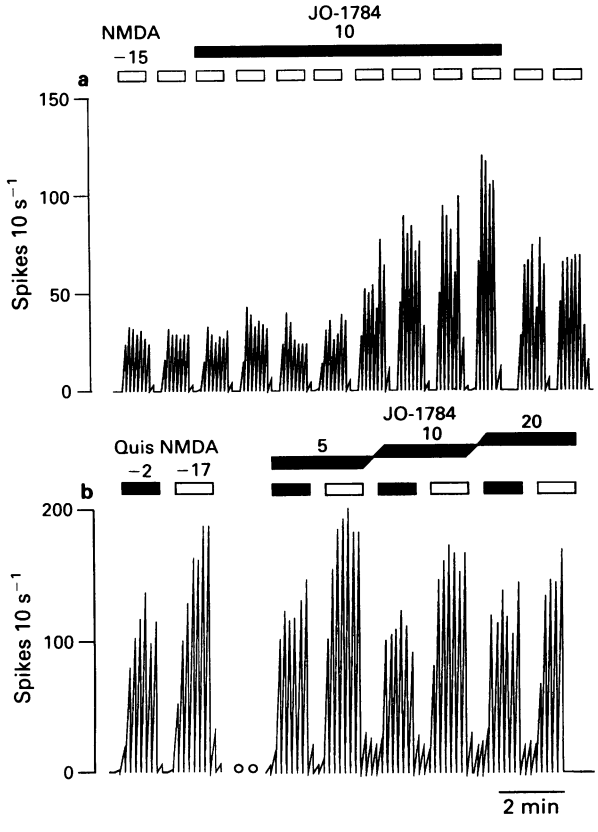

Figure 1 Integrated firing rate histograms of CA_3 dorsal hippocampus pyramidal neurones showing the effects of microiontophoretic applications of *N*-methyl-D-aspartate (NMDA) and quisqualate (Quis) before and during the microiontophoretic application of JO-1784 in a control rat (a) and in a rat pretreated with pertussis toxin (b). Bars indicate the duration of applications for which currents are given in nA and dots correspond to 10–15 min interruption, of the trace, in this and subsequent figures. Time base applies to both traces.

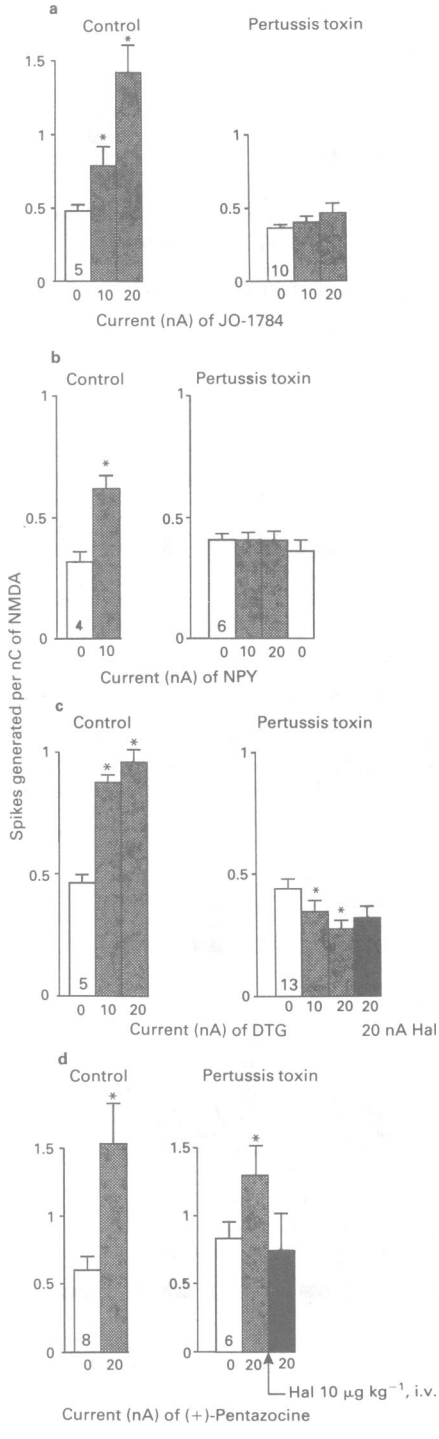

Figure 2 Responsiveness expressed as the number of spikes generated per nanocoulomb (nC; mean ± s.e.mean) of CA_3 dorsal hippocampus neurones to microiontophoretic applications of *N*-methy-D-aspartate (NMDA) before (open column), during (stippled columns) microiontophoretic applications of JO-1784 (a), neuropeptide Y (NPY), (b), 1,3-di(2-tolyl)guanidine (DTG) (c) and (+)-pentazocine (d), and following the microiontophoretic application or the intravenous administration (solid column) of haloperidol in control and pertussis toxin treated rats. The number at the base of the first column of each histogram in this and in subsequent figures indicates the number of neurones tested. In all series of experiments, the same neurones were recorded from during the complete sequence. All applications of NMDA and Quis were of 50 s. $*P<0.01$.

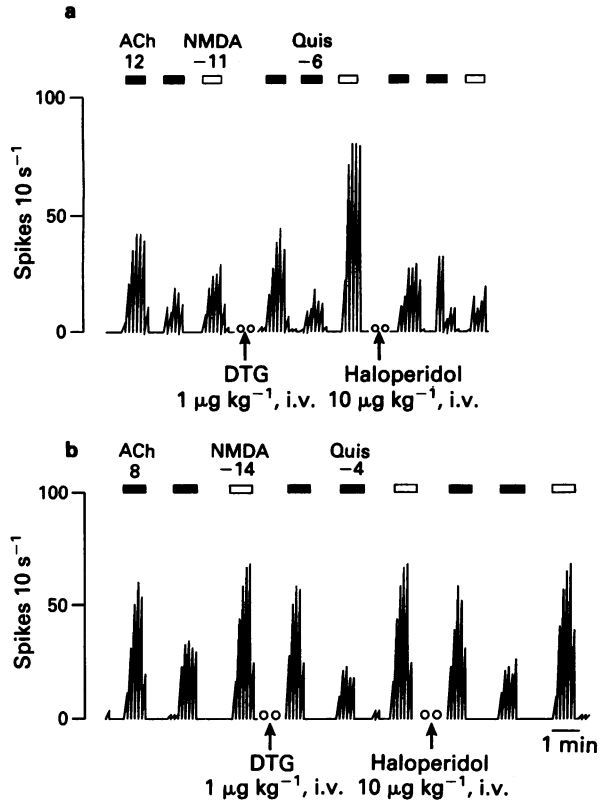

Figure 3 Integrated firing rate histograms showing the response of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic applications of acetylcholine (ACh), *N*-methyl-D-aspartate (NMDA) and quisqualate (Quis) before and following the intravenous administration of 1,3-di(2-tolyl)guanidine (DTG) and following the intravenous injection of haloperidol, in a control (a) and a pertussis toxin treated rat (b).

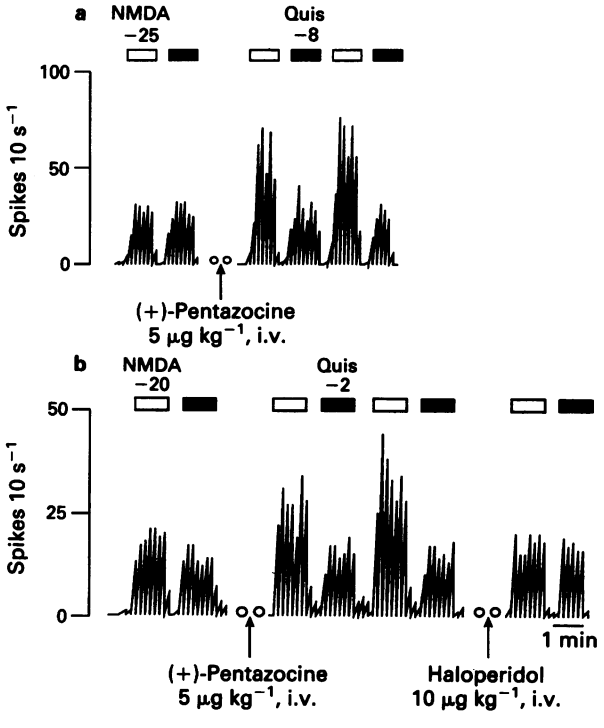

Figure 4 Integrated firing rate histograms showing the response of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic applications of *N*-methyl-D-aspartate (NMDA) and quisqualate (Quis) before and following the intravenous administration of (+)-pentazocine and following the intravenous injection of haloperidol, in a control (a) and a pertussis toxin treated rat (b).

DTG-induced attenuation of the NMDA response obtained in PTX-treated rats was not reversed by haloperidol (Figure 2c).

Effects of the intravenous administrations of DTG and (+)-pentazocine

In a second series of experiments, the two sigma ligands DTG and (+)-pentazocine, which were found to modulate the NMDA response when applied by microiontophoresis in PTX-treated rats, were administered intravenously. As illustrated in Figures 3a and 4a, DTG, at a dose of 1 $\mu g\,kg^{-1}$, and (+)-pentazocine, at a dose of 5 $\mu g\,kg^{-1}$, selectively enhanced by three fold the firing activity of CA_3 pyramidal neurones to microiontophoretic applications of NMDA in control rats (Figure 5), consistent with previous reports (Monnet *et al.*, 1990; 1992b). When injected in PTX-treated rats, DTG (1 $\mu g\,kg^{-1}$, i.v.) neither enhanced nor reduced the NMDA-induced neuronal activation (Figures 3b, 5b). However, in PTX-treated rats, (+)-pentazocine (5 $\mu g\,kg^{-1}$, i.v.) produced a marked and selective potentiation of the NMDA response, similar to that obtained in the control animals (Figures 4, 5a). The subsequent intravenous administration of a low dose of haloperidol (10 $\mu g\,kg^{-1}$) completely abolished the (+)-pentazocine-induced potentiation of the NMDA-induced firing activity of CA_3 pyramidal neurones in PTX-treated rats (Figures 4b, 5a). Neither the intravenous administration of DTG nor (+)-pentazocine modified the

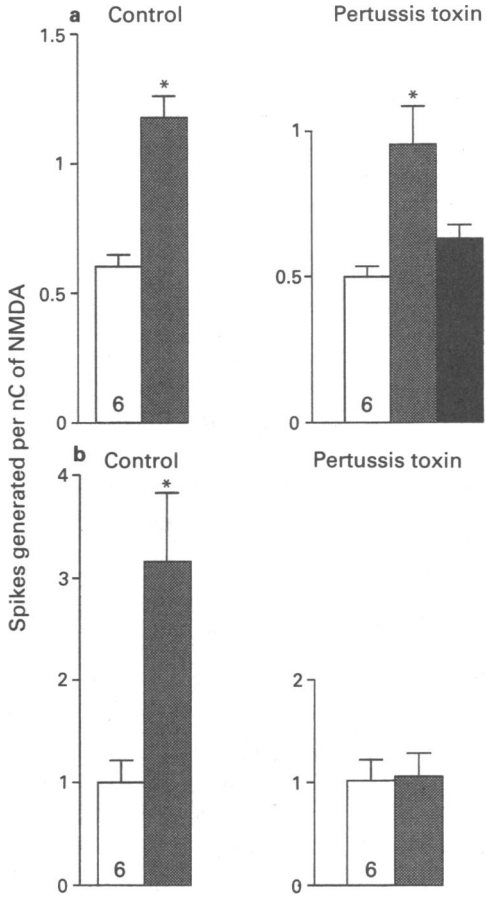

Figure 5 Responsiveness expressed as the number of spikes generated per nanocoulomb (mean ± s.e.mean) of CA_3 dorsal hippocampus neurones to microiontophoretic applications of *N*-methy-D-aspartate (NMDA) before (open columns) and after (stippled columns) the intravenous administration of (+)-pentazocine, 5 $\mu g\,kg^{-1}$ (a) and 1,3-di(2-tolyl)guanidine, (DTG) 1 $\mu g\,kg^{-1}$ (b). Since (+)-pentazocine still induced a potentiation of NMDA response in pertussis toxin-treated rats, a subsequent injection of haloperidolol (10 $\mu g\,kg^{-1}$, i.v.) was administered (solid column).

neuronal activation induced by microiontophoretic applications of Quis or ACh in control and PTX-treated rats (Figures 3–5).

Discussion

The present results obtained *in vivo*, in the CA_3 region of the rat dorsal hippocampus, show that the inactivation of $G_{i/o}$ proteins by PTX abolished the potentiating effects of DTG, JO-1784 and NPY on the NMDA response, but not that of (+)-pentazocine. In addition, in PTX-treated rats, haloperidol reversed the potentiating effect of (+)-pentazocine on the NMDA response.

The unaltered effects of NMDA and Quis on the firing activity of CA_3 pyramidal neurones, following $G_{i/o}$ protein inactivation by a PTX pretreatment, are consistent with previous observations in rat striatal neurones (Sladeczek *et al.*, 1985), cerebellar granule cells (Nicoletti *et al.*, 1986), hippocampal slices (Beaudry *et al.*, 1986) and forebrain synaptosomes (Recaens *et al.*, 1987) suggesting that the excitatory effects of these amino acids are not mediated by G proteins.

The observation that microiontophoretic applications of DTG, (+)-pentazocine, JO-1784 and NPY, as well as the intravenous administration of low doses of DTG and (+)-pentazocine, potentiated NMDA-induced neuronal activation of CA_3 dorsal hippocampus in control animals is consistent with previous *in vivo* studies (Monnet *et al.*, 1990; 1992b; Martin *et al.*, 1992) and with the data obtained by Iyengar *et al.* (1990b, 1991), who demonstrated that several sigma ligands also potentiated NMDA-dependent adrenocorticotropic hormone and prolactin release in pituitary cells as well as dopamine turnover in the striatum and olfactory tubercles.

The lack of effect of PTX-treatment on (+)-pentazocine-induced potentiation of the NMDA response (Figures 2, 4 and 5) suggests that this sigma ligand activates a subtype of sigma receptor not coupled to $G_{i/o}$ proteins. Haloperidol is known to bind with high affinity to dopamine, α_1-adrenoceptor, 5-HT, muscarinic and sigma receptors (Burt *et al.*, 1977; Peroutka *et al.*, 1977; Su, 1982). However, the only binding sites that haloperidol, DTG, JO-1784 and (+)-pentazocine share are the sigma sites (Su, 1982; Weber *et al.*, 1986; Roman *et al.*, 1990). Therefore, the suppression by haloperidol of the potentiating effect of (+)-pentazocine on the NMDA response in the present series of experiments constitutes conducive evidence that (+)-pentazocine potentiates the NMDA response by activating sigma receptors. These results differ from those of Itzhak (1989) who found that the binding of racemic pentazocine to sigma sites labelled with [^{3}H]-3-PPP was altered by GTP and Gpp(NH)p, suggesting that these sites were coupled to G proteins. Furthermore, (+)-pentazocine is considered to bind selectively to σ_1 receptors which have been suggested to be associated with a $G_{i/o}$ protein (Quirion *et al.*, 1992). However, De-Haven-Hudkins *et al.* (1992) have reported that the binding of [^{3}H]-(+)-pentazocine was insensitive to GTP and Gpp(NH)p, suggesting that [^{3}H]-(+)-pentazocine binds to sigma receptors not coupled to $G_{i/o}$ proteins. The apparently discrepant data of these two binding studies could be explained by the different forms of pentazocine and/or species used (guinea-pigs vs rats). The present results, in keeping with the observations of De Haven-Hudkins *et al.* (1992), suggest that the potentiating effect of (+)-pentazocine on the NMDA response is mediated by a subtype of σ_1 receptor not coupled to a $G_{i/o}$ protein and sensitive to haloperidol.

The high affinity sigma ligand, JO-1784, as well as NPY, have previously been shown to enhance the NMDA response *in vivo* as well as *in vitro* (Riviere *et al.*, 1990; Roman *et al.*, 1991b; Monnet *et al.*, 1992a,b,d), the effect of NPY being mediated via a non-Y_1, non-Y_2, non-Y_3 receptor, probably corresponding to a subtype of sigma receptor (Monnet *et al.*, 1992c,d). The similarity of some of the effects of JO-1784 and NPY has been reported in other models (Riviere *et al.*, 1990; 1993; Roman *et al.*, 1991a,b; Gué *et al.*, 1992a,b; Pascaud *et al.*, 1993). Thus, the prevention by PTX pretreatment of the potentiating effect of JO-1784 and NPY (Figure 1 and 2) is fully consistent with previous observations suggesting that they exert their effects on the NMDA response via a subtype of sigma receptor coupled to $G_{i/o}$ proteins (Junien *et al.*, 1991; Monnet *et al.*, 1992a).

Following the inactivation of $G_{i/o}$ proteins by PTX, the intravenous administration of DTG did not modify the neuronal response to NMDA and Quis, whereas the microiontophoretic application of DTG produced a slight but significant reduction of the excitatory effect of NMDA, but not of Quis, (Figures 2, 3 and 5). At present, we do not have a definite explanation for the differential effects of DTG when applied by microiontophoresis and intravenously. The suppression of the potentiating effect of DTG following PTX is consistent with the results obtained with JO-1784 and NPY. However, in addition, PTX pretreatment unveiled a second component ($G_{i/o}$ protein-insensitive) of the effect of DTG on the NMDA response, consisting of reduction in the NMDA response. Some reports have described an inhibitory effect of high doses of sigma ligands on NMDA-induced effects, albeit to a weaker degree than phencyclidine (PCP)-like drugs (Anis *et al.*, 1983; Lodge & Anis, 1984; Malouf *et al.*, 1988). This inhibitory effect of sigma ligands has been ascribed, however, to their low affinity for the PCP binding site (Lodge *et al.*, 1988; Malouf *et al.*, 1988; Church & Lodge, 1990; Monnet *et al.*, 1992b). Two groups have reported an inhibitory effect of DTG at low doses. First, Roth *et al.* (1993) have observed in the rat prepiriform cortex that concentrations of DTG as low as 1 nM produced an anticonvulsant effect, via a reduction of the NMDA response (Roth *et al.*, 1993). Second, Connick *et al.* (1992a) reported a reduction of the NMDA response by low doses of DTG in the CA_1 region of the rat dorsal hippocampus. One intriguing finding here is that the suppressant effect of DTG was not reversed by haloperidol (Figure 2c). In this and in previous experiments, haloperidol consistently suppressed the potentiating effects of sigma ligands. The reversal of the effects of (+)-pentazocine, DTG and JO-1784 by haloperidol has also been observed in the peripheral nervous system (Massamiri & Duckles, 1990), in behavioural models (Tam *et al.*, 1988; Steinfels *et al.*, 1989) as well as in the central nervous system both *in vivo* (Tam *et al.*, 1988; Monnet *et al.*, 1990; 1992b) and *in vitro* (Roman *et al.*, 1989; Connor *et al.*, 1992). Hence, since DTG and haloperidol both bind to both σ_1 and σ_2 receptors (Quirion *et al.*, 1992), it is possible that the suppressant effect of DTG on the NMDA responses in PTX-treated rats might not be ascribed to sigma receptors. However, some reports have provided evidence for the existence of more than two subtypes of sigma receptors. Zhou & Musacchio (1991) have suggested the existence of at least four subtypes of sigma receptors, one of them, R_4 having a moderately low affinity for DTG and very low affinity for haloperidol. Connick *et al.* (1992b), have proposed that this R_4 subtype of sigma receptor might not be coupled to a G protein. Moreover, the anticonvulsant effect of low doses of DTG is also insensitive to haloperidol (Roth *et al.*, 1993). Therefore, the observation that, in PTX-treated rats, the DTG-induced reduction of the NMDA response was not reversed by haloperidol (Figure 2c) raises the possibility that DTG might exert its inhibitory effect via a haloperidol-insensitive sigma receptor not coupled to $G_{i/o}$ protein, possibly corresponding to the R_4 subtype.

In conclusion, the present results provide further support for the notion that several types of sigma receptors exist in the central nervous system and that some sigma receptors are coupled to $G_{i/o}$ proteins.

We wish to thank T. Vo for computer programming, statistical analysis and illustrations, and L. Martin for her secretarial assis-

tance. This work was supported, in part, by the Medical Research Council of Canada, by the Royal Victoria Hospital Research Institute and by the Fonds de la Recherche en Santé du Québec (F.R.S.Q.). F.P.M. was in receipt of a Convention Industrielle de la Formation par la Recherche Fellowship from the Ministère de la Santé, France. G.D. and R.B. are in receipt of a Scholarship and a Fellowship from the F.R.S.Q., respectively and B.G. of a Fellowship from the Fondation pour la Recherche Médicale.

References

ANDRADE, R., MALENKA, R.C. & NICOLL, R.A. (1986). A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. *Science*, **234**, 1261–1265.

ANIS, A., BERRY, S.C., BURTON, N.R. & LODGE, D. (1983). The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *J. Pharmacol. Exp. Ther.*, **79**, 565–575.

BEART, P.M., OSHEA, R.D. & MANALLAK, D.T. (1989). Regulation of sigma-receptors – high-affinity and low-affinity agonist states, GTP shifts, and up-regulation by rimcazole and 1,3-di(2-tolyl)guanidine. *J. Neurochem.*, **53**, 779–788.

BEAUDRY, M., EVANS, J. & LYNCH, G. (1986). Excitatory amino acids inhibit stimulation of phosphatidylinositol metabolism by aminergic agonists in hippocampus. *Nature*, **319**, 329–331.

BLIER, P., LISTA, A. & DE MONTIGNY, C. (1992). Differential properties of pre- and postsynaptic 5-hydroxytryptamine$_{1A}$ receptors in the dorsal raphe and hippocampus: II. Effect of pertussis and cholera toxins. *J. Pharmacol. Exp. Ther.*, **265**, 16–23.

BOUCHARD, P., DUMONT, Y., FOURNIER, A., ST-PIERRE, S. & QUIRION, R. (1993). Evidence for in vivo interactions between neuropeptide Y-related peptides and sigma receptors in the mouse hippocampal formation. *J. Neurosci.*, **13**, 3926–3931.

BROOKS, P.A., KELLY, J.S., ALLEN, J.M., SMITH, D.A.S. & STONE, T.W. (1987). Direct excitatory effects of neuropeptide Y (NPY) on rat hippocampal neurons *in vitro*. *Brain Res.*, **408**, 295–298.

BURT, D.R., CREESE, I. & SNYDER, S.H. (1977). Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. *Science*, **196**, 326–328.

CHATTARJI, S., STANTON, P.K. & SEJNOWSKI, T.J. (1989). Commissural synapses, but not mossy fiber synapses, in hippocampal field CA_3 exhibit associative long-term potentiation and depression. *Brain Res.*, **495**, 145–150.

CHURCH, J. & LODGE, D. (1990). Failure of sigma-receptor ligands to reduce the excitatory actions of N-methyl-DL-aspartate on rat spinal neurons *in vivo*. *J. Pharm. Pharmacol.*, **42**, 56–57.

CONNICK, J.H., ADDAE, J.I., NICHOLSON, C.D. & STONE, T.W. (1992a). The σ ligand 1,3-di-o-tolyguanidine depresses amino acid-induced excitation non-selectively in rat brain. *Eur. J. Pharmacol.*, **214**, 169–173.

CONNICK, J.H., HANLON, G., ROBERTS, J., FRANCE, L., FOX, P.K. & NICHOLSON, C.D. (1992b). Multiple σ binding sites in guinea-pig and rat brain membranes: G-protein interactions. *Br. J. Pharmacol.*, **107**, 726–731.

CONNOR, M., PATTERSON, T. & CHAVKIN, C. (1992). Zinc is a potential endogenous ligand for one form of the haloperidol-sensitive sigma receptor. *Soc. Neurosci. Abst.*, **18**, 195.6.

DEHAVEN-HUDKINS, D.L., FLEISSNER, L.C. & FORD-RICE, F.Y. (1992). Characterization of the binding of [^{3}H](+)-pentazocine to σ recognition sites in guinea pig brain. *Eur. J. Pharmacol.: Mol. Pharmacol.*, **227**, 371–378.

FREDHOLM, B.B. & LINDGREN, E. (1988). Effects of N-ethylmaleimide and forskolin on noradrenaline release from rat hippocampal slices. Evidence that prejunctional adenosine and α-receptors are linked to N-proteins but not adenylate cyclase. *Acta Physiol. Scand.*, **130**, 95–105.

GILMAN, A.G. (1987). G proteins: Transducers of receptor-generated signals. *Annu. Rev. Biochem.*, **56**, 615–649.

GUÉ, M., JUNIEN, J.L., DEL RIO, C. & BUENO, L. (1992a). Neuropeptide Y and sigma ligand (JO 1784) suppress stress-induced colonic motor disturbances in rats through sigma and cholecystokinin receptors. *J. Pharmacol. Exp. Ther.*, **261**, 850–855.

GUÉ, M., YONEDA, M., MÖNNIKES, H., JUNIEN, J.-L. & TACHÉ, Y. (1992b). Central neuropeptide Y and the sigma ligand, JO 1784, reverse corticotropin-releasing factor-induced inhibition of gastric acid secretion in rats. *Br. J. Pharmacol.*, **107**, 642–647.

HERTTING, G. & ALLGAIER, C. (1988). Participation of protein kinase C and regulatory G proteins in modulation of the evoked noradrenaline release in brain. *Cell Mol. Neurobiol.*, **8**, 105–114.

ITZHAK, Y. (1989). Multiple affinity binding states of the sigma-receptor effect of GTP-binding protein-modifying agents. *Mol. Pharmacol.*, **36**, 512–517.

ITZHAK, Y. & KHOURI, M. (1988). Regulation of the binding of sigma- and phencyclidine (PCP)-receptor ligands in rat brain membranes by guanine nucleotides and ions. *Neurosci. Lett.*, **85**, 147–152.

IYENGAR, S., DILWORTH, V.M., MICK, S.J., CONTRERAS, P.C., MONAHAN, J.B., RAO, T.S. & WOOD, P.L. (1990a). Sigma receptors modulate both A9 and A10 dopaminergic neurons in the rat brain – functional interaction with NMDA receptors. *Brain Res.*, **524**, 322–326.

IYENGAR, S., MICK, S., DILWORTH, V., MICHEL, J., RAO, T.S., FARAH, J.M. & WOOD, P.L. (1990b). Sigma receptors modulate the hypothalamic-pituitary-adrenal (Hpa) axis centrally - evidence for a functional interaction with NMDA receptors, *in vivo*. *Neuropharmacology*, **29**, 299–303.

IYENGAR, S., WOOD, P.L., MICK, S.J., DILWORTH, V.M., GRAY, N.M., FARAH, J.M., RAO, T.S. & CONTRERAS, P.C. (1991). (+)-3-[3-Hydroxyphenyl-*N*-(1-propyl) piperidine] selectively differentiates effects of sigma ligands on neurochemical pathways modulated by sigma receptors. Evidence for subtypes, *in vivo*. *Neuropharmacology*, **30**, 915–922.

JUNIEN, J.L., GUÉ, M. & BUENO, L. (1991). Neuropeptide Y and sigma ligand (JO 1784) act through a Gi protein to block the psychological stress and corticotropin-releasing factor-induced colonic motor activation in rats. *Neuropharmacology*, **30**, 1119–1124.

LODGE, D. & ANIS, N.A. (1984). Effects of ketamine and three other short acting anaesthetics on spinal reflexes and inhibitions in the cat. *Br. J. Anaesth.*, **56**, 1143–1151.

LODGE, D., ARAM, J.A., CHURCH, J., DAVIES, S.N., FLETCHER, E. & MARTIN, D. (1988). Electrophysiological studies of the interaction between phencyclidine/sigma receptor agonists and excitatory amino acid neurotransmission on central mammalian neurons. In *Sigma and Phencyclidine-like Compounds as Molecular Probes in Biology*. ed. Domino, E.F. & Kamenka, J.M. pp. 239–250. Ann Arbor: NPP Books.

MALOUF, A.T., SWEARENGEN, E. & CHAVKIN, C. (1988). Comparison of the actions of phencyclidine and sigma ligands on CA1 hippocampal pyramidal neurons in the rat. *Neuropharmacology*, **27**, 1161–1170.

MARTIN, W.J., ROTH, J.S. & WALKER, J.M. (1992). The effects of sigma compounds on both NMDA- and non NMDA-mediated neuronal activity in rat hippocampus. *Soc. Neurosci. Abst.*, **18**, 16.6.

MASSAMIRI, T. & DUCKLES, S.P. (1990). Multiple vascular effects of sigma-ligands and PCP ligands-inhibition of amine uptake and contractile responses. *J. Pharmacol. Exp. Ther.*, **253**, 124–129.

MONNET, F.P., DEBONNEL, G., JUNIEN, J.L. & DE MONTIGNY, C. (1990). *N*-methyl-D-aspartate-induced neuronal activation is selectively modulated by sigma receptors. *Eur. J. Pharmacol.*, **179**, 441–445.

MONNET, F.P., BLIER, P., DEBONNEL, G. & DE MONTIGNY, C. (1992a). Modulation by sigma ligands of *N*-methyl-D-aspartate-induced [^{3}H]noradrenaline release in the rat hippocampus: G-protein dependency. *Naunyn-Schmied. Arch. Pharmacol.*, **346**, 32–39.

MONNET, F.P., DEBONNEL, G. & DE MONTIGNY, C. (1992b). *In vivo* electrophysiological evidence for a selective modulation of *N*-methyl-D-aspartate-induced neuronal activation in rat CA_3 dorsal hippocampus by sigma ligands. *J. Pharmacol. Exp. Ther.*, **261**, 123–130.

MONNET, F.P., DEBONNEL, G., FOURNIER, A. & DE MONTIGNY, C. (1992c). Neuropeptide Y potentiates the *N*-methyl-D-aspartate response in the CA_3 dorsal hippocampus. II. Involvement of a subtype of sigma receptor. *J. Pharmacol. Exp. Ther.*, **263**, 1219–1225.

MONNET, F.P., FOURNIER, A., DEBONNEL, G. & DE MONTIGNY, C. (1992d). Neuropeptide Y potentiates selectively the *N*-methyl-D-aspartate response in the rat CA_3 dorsal hippocampus. I. Involvement of an atypical neuropeptide Y receptor. *J. Pharmacol. Exp. Ther.*, **263**, 1212–1218.

NICOLETTI, F., IADAROLA, M.J., WROBLEWSKI, J.T. & COSTA, E. (1986). Excitatory amino acids recognition sites-coupled with inositol phospholipid metabolism: developmental changes and interaction with α_1-adrenoceptors. *Proc. Natl. Acad. Sci. U.S.A.*, **83,** 1931–1934.

PASCAUD, X.B., CHOVET, M., ROZE, C. & JUNIEN, J.-L. (1993). Neuropeptide Y and σ receptor agonists act through a common pathway to stimulate duodenal alkaline secretion in rats. *Eur. J. Pharmacol.*, **231,** 389–394.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. 2nd edition. San Diego, CA: Academic press.

PEROUTKA, S.J., U'PRICHARD, D.C., GREENBERG, D.A. & SNYDER, S.H. (1977). Neuroleptic drug interactions with norepinephrine α receptor binding sites in rat brain. *Neuropharmacology*, **16,** 549–556.

QUIRION, R., BOWEN, W.D., ITZHAK, Y., JUNIEN, J.L., MUSACCHIO, J.M., ROTHMAN, R.B., SU, T.P., TAM, S.W. & TAYLOR, D.P. (1992). Classification of sigma binding sites: a proposal. In *Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Neuroprotection*? ed. Kamenka, J.M. & Domino, E.F. pp. 927–933. Ann Arbor: NPP Books.

RECAENS, M., SASSETTI, I., NOURIGAT, A., SLADECZEK, F. & BOCKAERT, J. (1987). Characterization of subtypes of excitatory amino acid receptors involved in the stimulation of inositol phosphate synthesis in rat brain synaptosomes. *Eur. J. Pharmacol.*, **141,** 87–93.

RIVIERE, P.J.M., PASCAUD, X., JUNIEN, J.L. & PORRECA, F. (1990). Neuropeptide-Y and JO 1784, a selective sigma ligand, alter intestinal ion transport through a common, haloperidol-sensitive site. *Eur. J. Pharmacol.*, **187,** 557–559.

RIVIERE, P.J.M., RAO, R.K., PASCAUD, X., JUNIEN, J.L. & PORRECA, F. (1993). Effects of neuropeptide Y, peptide YY and sigma ligands on ion transport in mouse jejunum. *J. Pharmacol. Exp. Ther.*, **264,** 1268–1274.

ROMAN, F.J., PASCAUD, X., DUFFY, O., VAUCHE, D., MARTIN, B. & JUNIEN, J.L. (1989). Neuropeptide-Y and peptide – YY interact with rat brain sigma and PCP binding sites. *Eur. J. Pharmacol.*, **174,** 301–302.

ROMAN, F.J., PASCAUD, X., DUFFY, O. & JUNIEN, J.L. (1991a). *N*-methyl-D-aspartate receptor complex modulation by neuropeptide Y and peptide YY in rat hippocampus *in vitro*. *Neurosci. Lett.*, **122,** 202–204.

ROMAN, F.J., PASCAUD, X., DUFFY, O. & JUNIEN, J.L. (1991b). Modulation by neuropeptide Y and peptide YY of NMDA effects in hippocampal slices: Role of sigma receptors. In *NMDA Receptor Related Agents: Biochemistry, Pharmacology and Behavior* ed. Kameyama, T., Nabeshima, T. & Domino, E.F. pp. 211–218. Ann Arbor: NPP Books.

ROMAN, F.J., MARTIN, B. & JUNIEN, J.L. (1993). In vivo interaction of neuropeptide Y and peptide YY with σ receptor sites in the mouse brain. *Eur. J. Pharmacol.*, **242,** 305–307.

ROMAN, F.J., PASCAUD, X., MARTIN, B., VAUCHE, D. & JUNIEN, J.L. (1990). JO-1784, a potent and selective ligand for rat and mouse brain sigma sites. *J. Pharm. Pharmacol.*, **42,** 439–440.

ROTH, J.E., FRANKLIN, P.H. & MURRAY, T.F. (1993). The σ receptor ligand 1,3-di(2-tolyl)guanidine is anticonvulsant in the rat prepiriform cortex. *Eur. J. Pharmacol.*, **236,** 327–331.

SLADECZEK, F., PIN, J.P., RECAENS, M., BOCKAERT, J. & WEISS, S. (1985). Glutamate stimulates inositol phosphate formation in striatal neurons. *Nature*, **317,** 717–719.

STEINFELS, G.F., TAM, S.W. & COOK, L. (1989). Electrophysiological effects of selective sigma-receptor agonists, antagonists, and the selective phencyclidine receptor agonist MK-801 on midbrain dopamine neurons. *Neuropsychopharmacology*, **2,** 201–208.

SU, T.P. (1982). Evidence for sigma opioid receptor: binding of [^{3}H]SKF-10047 to etorphine-inaccessible sites in guinea-pig brain. *J. Pharmacol. Exp. Ther.*, **223,** 284–290.

TAM, S.W., STEINFELS, G.F. & COOK, L. (1988). Biochemical and behavioural aspects of sigma and phencyclidine receptors: Similarities and differences. In *Sigma and Phencyclidine-like Compounds as Molecular Probes in Biology*. ed. Domino, E.F. & Kamenka, J.-M. pp. 383–396. Ann Arbor: NPP Books.

WEBER, E., SONDERS, M., QUARUM, M., MCLEAN, S., POU, S. & KEANA, J.F.W. (1986). 1,3-Di(2-[5-3H]tolyl)guanidine: a selective ligand that labels sigma-type receptors for psychotomimetic opiates and antipsychotic drugs. *Proc. Natl. Acad. Sci. U.S.A.*, **83,** 8784–8788.

ZHOU, G.Z. & MUSACCHIO, J.M. (1991). Computer-assisted modelling of multiple dextromethrophan and sigma binding sites in guinea pig brain. *Eur. J. Pharmacol: Mol. Pharmacol.*, **206,** 261–269.

(Received December 10, 1993
Revised March 2, 1994
Accepted March 8, 1994)