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Failure of σ-receptor ligands to reduce the excitatory actions of *N*-methyl-DL-aspartate on rat spinal neurons in-vivo

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Abstract—Haloperidol and (+)-3-PPP, compounds with known affinity for the σ-receptor, have been examined for their ability to reduce the excitatory actions of *N*-methyl-DL-aspartate (NMDLA), quisqualate and kainate on rat spinal neurons in-vivo. The actions of (−)-3-PPP were also tested. Haloperidol was injected intravenously whereas the 3-PPP enantiomers were administered by microelectrophoresis. Haloperidol had little effect on excitations evoked by NMDLA, quisqualate or kainate whereas both (+)- and (−)-3-PPP usually enhanced, non-selectively, responses to all three excitatory amino acid analogues. The results support suggestions that phencyclidine (PCP)-like compounds with affinity for both PCP and σ-receptors reduce neuronal excitations mediated by the *N*-methyl-D-aspartate (NMDA) receptor via a selective effect at the PCP site.

It is now apparent that there are two distinct central binding sites for PCP and PCP-like drugs (Quirion et al 1987). The PCP receptor is labelled preferentially by MK-801, the thienyl derivative of PCP (TCP) and by PCP itself. In contrast, the σ-receptor displays a relatively low affinity for these compounds but high affinity for haloperidol, (+)-3-[3-hydroxyphenyl]-*N*-(1-propyl)piperidine ((+)-3-PPP) and 1,3-di-*o*-tolylguanidine (DTG) (Largent et al 1984; Weber et al 1986). In addition, the σ site shows a relatively greater affinity for the (+)-isomers of certain benzomorphans, including cyclazocine and *N*-allylnormetazocine (NANM), than the PCP site.

In recent years it has become clear that a good correlation exists between the relative ability of a series of drugs with PCP-like activity to displace tritiated ligands from the PCP receptor and to selectively and non-competitively reduce neuronal excitations mediated by the NMDA subclass of excitatory amino acid receptor (see Lodge et al 1988). Nevertheless, it is also apparent that some compounds with only low affinity for the PCP receptor, but high affinity for the σ-receptor (e.g. (+)-pentazocine), are still able to reduce NMDA-evoked excitations in-vivo, albeit weakly. It therefore became important to examine the effects of compounds with even greater selectivity for the σ-receptor than, say, (+)-pentazocine in the same in-vivo system which we have previously used to explore the NMDA antagonist actions of a range of PCP-like compounds (Lodge et al 1988). Accordingly, we have tested the effects of (+)-3-PPP and haloperidol on rat spinal neuronal excitations evoked by the excitatory amino acid analogues NMDLA, quisqualate and kainate. We have also examined the actions of (−)-3-PPP, which has between a 5- and 10-fold lower affinity for the σ-receptor than its enantiomer, depending upon which tritiated ligand is used to label the receptor (see Largent et al 1984).

Materials and methods

Experiments were performed on pentobarbitone-anaesthetized Wistar rats, as described in detail elsewhere (Anis et al 1983). Multibarrel glass electrodes were used to record extracellular action potentials of single spinal neurons and to administer NMDLA Na (200 mM; pH 8·1), quisqualate Na (5 mM in 200 mM NaCl; pH 8·0) and kainate Na (5 mM in 200 mM NaCl; pH 8·2) by microelectrophoresis. The firing rate of the neuron under study was monitored continuously during the sequential ejection of two or more excitants with current magnitude and duration adjusted to give approximately equal and submaximal responses in the control period. The test compounds were then applied, either by intravenous injection (haloperidol) or by microelectrophoresis ((+)- and (−)-3-PPP), and the change in peak firing frequency to each excitant was noted. Both (+)- and (−)-3-PPP (HCl salts) were ejected from a 50 mM in 150 mM NaCl solution (pH 4·5). Haloperidol was initially dissolved with the aid of a small amount of lactic acid. (+)- and (−)-3-PPP were generous gifts from Dr A. L. Gundlach and haloperidol was obtained from the Sigma Chemical Co.

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Results

The effects of (+)- and (−)-3-PPP were directly compared on 18 neurons (Table 1). With neither compound was there any suggestion of selective antagonism of NMDLA or the other amino acids, even at ejection currents which were high enough (>80 nA) to produce marked decreases in action potential amplitude. Indeed, as shown in Fig. 1, both compounds usually (13 out of 18 comparisons) enhanced non-selectively responses to all the amino acid excitants. Similarly, (+)-3-PPP augments NMDA-evoked excitations on hippocampal CA1 pyramidal neurons in-vitro (Malouf et al 1988). Although not shown, the effects of microelectrophoretically-applied (+)-3-PPP were directly compared with those of ketamine on four neurons; only ketamine produced a selective reduction in NMDLA-evoked responses, as previously described (Anis et al 1983).

The effects of haloperidol were examined on six neurons. In cumulative doses ranging from 0·1 to 2·0 mg kg^{-1}, responses to NMDLA, quisqualate and kainate were minimally affected; the maximum depressions observed were 8, 10 and 5%, respectively. In addition, these small reductions in responses were transient, being apparent for only 3 to 5 min following the injection of the drug. In contrast to (+)- and (−)-3-PPP, the amino acid-evoked excitations were never enhanced by haloperidol.

Discussion

The results demonstrate that (+)-3-PPP and haloperidol fail to reduce selectively the excitatory actions of NMDLA on rat spinal neurons in-vivo. Similarly, in-vivo, haloperidol has no effect on nociceptive spinal reflexes (which are mediated, at least in part, by NMDA receptors), fails to affect the ability of either ketamine or MK-801 to reduce spinal nociceptive reflexes (Parsons et al 1988) and does not protect against NMDA-induced lethality (Leander et al 1988). In addition, haloperidol does not affect the NMDLA blocking actions of either cyclazocine or pentazocine in-vivo (Church & Lodge, unpublished observations), or of ketamine or NANM in-vitro (Coan & Collingridge 1987; Lodge et al 1988). Also in-vitro, neither (+)-3-PPP, DTG nor haloperidol antagonize NMDA-induced neuronal excitations or depolarizations (Lodge et al 1988; Malouf et al 1988; Aram et al 1989).

Table 1. The effects of (+)- and (−)-3-PPP on the excitation of rat spinal neurons by NMDLA, quisqualate and kainate.

	Ejection current (nA)		% change in responses to: NMDLA		quisqualate		kainate	
Drug	mean ± s.d.	range	mean ± s.d.	range	mean ± s.d.	range	mean ± s.d.	range
(+)-3-PPP	34 ± 9	20 to 50	+18 ± 21	+48 to −14	+14 ± 25	+48 to −35	+16 ± 20	+45 to −15
(−)-3-PPP	33 ± 8	20 to 40	+16 ± 8	+52 to −21	+17 ± 7	+32 to +4	+14 ± 15	+43 to −15

Ejection currents are given in nA (mean ± s.d. and range) and the effects of the test compounds on the action potential frequencies evoked by the excitants are given as a percentage change (mean ± s.d.) compared with the appropriate control value. The range of percentage change for each test compound against each agonist is also shown.

The results therefore support our previous conclusion, based on studies of the relative NMDA antagonist potencies of a series of compounds which had affinity for both σ and PCP receptors, that NMDA antagonism by PCP-like drugs is effected, not via the σ-receptor but via a specific interaction with the PCP site (see Lodge et al 1988). In addition, we have previously noted that there is a good correlation for PCP-like drugs between relative NMDA antagonist potency and relative potency for eliciting PCP-like discriminative stimulus effects, suggesting in turn that NMDA antagonism may be involved in the transduction of the discriminative stimulus properties of PCP. The failure of haloperidol and (+)- and (−)-3-PPP to reduce the excitatory actions of NMDLA is consistent with the failure of haloperidol, DTG and the 3-PPP enantiomers to affect the discriminative stimulus properties of PCP (e.g. see Willetts & Balster 1988).

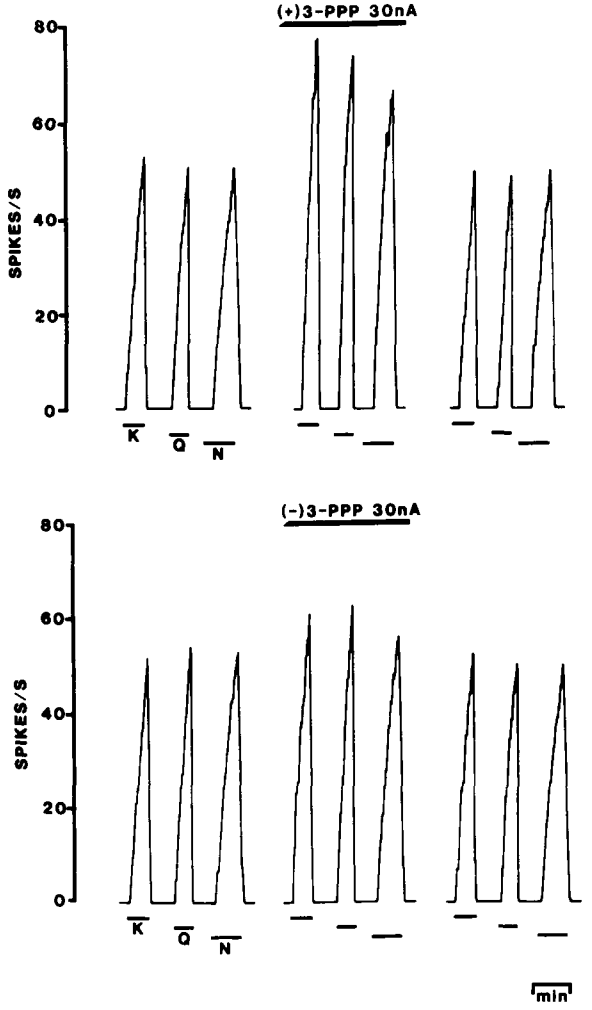

FIG. 1. The effects of (+)-3-PPP (upper traces) and (−)-3-PPP (lower traces), each ejected for 10 min at 30 nA, on the excitation of a rat spinal neuron by sequentially-administered kainate (K; 62 nA), quisqualate (Q; 34 nA) and NMDLA (N; 50 nA) as indicated by the bars beneath the traces. In both sets of records, the segment to the left shows responses immediately before the start of ejection of the test compounds and the segment to the right shows recovery after 20 min. Ordinate: firing rate in spikes sec^{-1}. Abscissa: time (calibration bar = 1 min).

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