EFFECTS OF PHENCYCLIDINE AND ITS DERIVATIVES ON ENTERIC NEURONES

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1   The effects of N-(1-phenylcyclohexyl) piperidine (PCP) and related drugs on isolated intact segments of the guinea-pig ileum were determined.

2   1-1-(2-Thienyl) cyclohexylpiperidine (TCP), PCP and ketamine decreased the height of electrically induced contractions (0.1 Hz) of intact segments of isolated guinea-pig ileum.

3   Thirty to forty percent of the inhibition of contraction height (0.1 Hz) was reversed by pretreatment with the pure narcotic antagonist, naloxone. This naloxone-reversible component showed cross-tolerance with morphine.

4   PCP pretreatment caused a shift to the right in the dose-response curve to acetylcholine (ACh) that was not parallel with the control dose-response curve. Thus PCP does not interact with the muscarinic cholinoceptor in a strictly atropine-like competitive fashion.

5   Binding sites for [³H]-PCP were detected in homogenates of the guinea-pig longitudinal muscle-myenteric plexus preparation.

6   The affinity constants and the rank order of potencies of various PCP derivatives competing with [³H]-PCP for binding suggest that these binding sites are very similar to those found in the central nervous system.

7   These data suggest that the guinea-pig isolated ileum may be used as an *in vitro* system for studying the mechanism of action of phencyclidines.

Introduction

Phencyclidine (N-(1-phenylcyclohexyl) piperidine; PCP), originally developed as a general anaesthetic, has potent psychotomimetic effects in man. This property has promoted its popularity amongst illicit users to the extent that it is currently the most widely abused drug in the United States.

Subanaesthetic doses of PCP in humans have profound effects on the central nervous system (CNS); these include the production of nystagmus, ataxic gait, muscle rigidity, characteristic electroencephalographic changes, stereotypy and psychotomimetic effects (Domino, 1964; Fauman & Fauman, 1978). PCP-induced psychotic symptoms are of particular interest in that they have a close resemblance to those of schizophrenia (Luby, Cohen, Rosenbaum, Gottlieb & Kelley, 1959). Thus, insight into the mode of action of PCP might provide some understanding of the neurochemical perturbations which underlie naturally occurring psychotic states.

Previous pharmacological analyses of the effects of PCP have indicated that these drugs inhibit the uptake of catecholamines and indoleamines by brain tissue (Taube, Montel, Haw & Starket, 1975; Garey & Heath, 1976; Smith, Meltzer, Arora & David, 1977), have anticholinergic properties and inhibit acetylcholinesterase (Maayani & Weinstein, 1979) and interact with both muscarinic and opiate receptor sites that are in the CNS (Vincent, Cavey, Kamenka, Geneste & Lazdunski, 1978). In addition, specific PCP binding sites in brain have recently been described (Vincent, Kartalovski, Geneste, Kamenka & Lazdunski, 1979; Zukin & Zukin, 1979). Thus, PCP and its derivatives may interact with several neuronal systems. However, it is difficult to establish how far removed a given PCP effect is from the site at which the CNS actions of the drug are initiated. We have sought a model system less complex than the CNS in which to study the actions of phencyclidine and related drugs. The isolated ileum of the guinea-pig is one such model system that has been used extensively, and with considerable success to study the CNS mechanism of action of opiates and should be a useful model to study mechanisms that mediate CNS responses to drugs in general.

This paper indicates that PCP and its derivatives interact with and affect several neuronal systems present in the enteric nervous system. In addition, it is suggested that this system might be suitable for studying the CNS mechanisms of action of these compounds.

Methods

[³H]-phencyclidine binding in guinea-pig ileum: tissue preparation

Male albino guinea-pigs (400–450 g) were killed by exsanguination. The distal small intestine was rapidly removed and placed in Krebs-Tris solution, of the following composition (mM): NaCl 118, KCl 4.75, CaGl₂ 2.54, KH₂PO₄ 1.10, MgSO₄ 1.2 and Tris 25; pH 7.4 (room temperature). The lumen of the intestine was flushed with a minimum of Krebs-Tris solution at room temperature and strips of longitudinal muscle with attached myenteric plexus (LM-MP) were prepared by the method of Ambache (1954). LM-MP preparations were stored in Krebs-Tris solution that was maintained at 4°C until tissue from 2 guinea-pigs had been processed. The strips were then blotted, weighed and minced with small scissors. The minced tissue was suspended in the above Krebs-Tris buffer (20 mg wet wt. per ml) and homogenized with a Brinkman Polytron homogenizer (setting no. 6, 1 min). The homogenate was centrifuged for 15 min (20,000 g, 4°C). The resulting pellet was washed once by centrifugation in 5 mM Tris (pH 7.4) and resuspended for assay in the original volume of 5 mM Tris by homogenization using the Polytron homogenizer (setting no. 6, 1 min) and then a Teflon-glass hand homogenizer (20 strokes).

Binding assays

For PCP binding, aliquots of freshly prepared LM-MP homogenate (1.0 ml, approximately 500 μg of protein) in 5 mM Tris-HCl buffer, pH 7.4, were incubated in triplicate at 4°C for 45 min with 7 nM [³H]-PCP (336,000 ct/min) alone or in the presence of 100 μM PCP or other drugs. Free ligand was separated from membrane-bound [³H]-PCP by filtration under reduced pressure through GF/B glass fibre filters (Whatman) which had been presoaked in 0.5% bovine serum albumin (BSA; Zukin & Zukin, 1981). The filters were rapidly washed with two aliquots of 10 ml of Tris-HCl 5 mM, pH 7.4, 4°C. Filters were then transferred to Aquasol-toluene (2:1, vol/vol) and assayed by liquid scintillation spectrometry (Intertechnique ABAL SL 40) at a counting efficiency of approximately 50%. Opiate receptor binding assays were performed as previously described (Zukin & Gintzler, 1980).

Physiological studies

Male albino guinea-pigs weighing 400–500 g were used. After the animal was exsanguinated the terminal portion of the ileum was removed and the last 10 cm were discarded. The lumen was flushed with a minimum of 10 ml of Krebs solution at room temperature and the preparation (4 cm) was mounted over the short end of a J-shaped glass tube which also contained a silver wire as a stimulating electrode. The mounted preparation was then placed into a 25 ml organ bath which was kept at 37°C. Resting tension was 1 g. The ileum was stimulated by transmural electrical stimulation essentially as described by Paton (1957) and modified by Gintzler & Musacchio (1975). Rectangular current pulses of 300 μs duration and of sufficient strength to produce a maximal response to a single shock were applied to the electrodes at the indicated frequency. The isometric contractions of the gut were recorded with a Grass FT03 transducer connected to a Brush polygraph. All of the experiments were carried out on pieces of ileum suspended in Krebs solutions of the following composition (mM): NaCl 118, KCl 4.7, CaCl₂.2H₂O 2.5, MgCl₂ 1.2, NaH₂PO₄.H₂O 1.2, NaHCO₃ 25 and glucose 11. The buffer was bubbled with a mixture of 95% O₂–5% CO₂ which maintained the pH at 7.4.

Tolerance/dependence formation

Morphine dependence was induced by the subcutaneous implantation of five morphine pellets (each containing 75 mg of morphine base; Berkowitz, Cerreta & Spector, 1974) under light ether anaesthesia. On the 4th day following pellet implantation the animal was killed and the gut was mounted as described above. Drugs used were: morphine sulphate (Mallinckrodt), acetylcholine chloride (Sigma), naloxone hydrochloride (Endo Laboratories), (–)-[piperidy-3, 4-³H] [N-(1-phenylcyclohexyl)-piperidine (60 Ci/mmol), [³H]-dihydromorphine (DHM) (38 Ci/mmol) and [³H]-D-Ala²-Met-enkephalinamide ([³H]-DALA; 40 Ci/mmol) (New England Nuclear). Non-radioactive PCP, 1-1-(2-thienyl)cyclohexyl-piperidine (TCP), N-ethyl-1-phenylcyclohexylamine (PCE), 1-(1-phenylcyclohexyl) pyrrolidine (PHP), 1-(1-phenylcyclohexyl) morpholine (PCM), and ketamine were generously supplied by the National Institute of Drug Abuse.
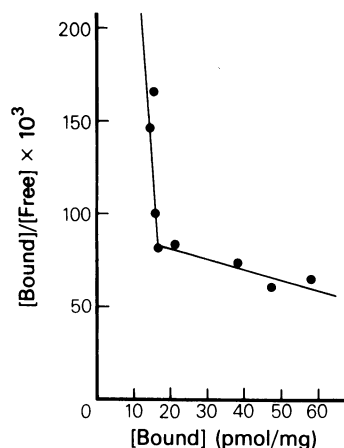
Results

[³H]-phencyclidine binding to longitudinal muscle-myenteric plexus homogenate

Specific [³H]-PCP binding was defined as the total
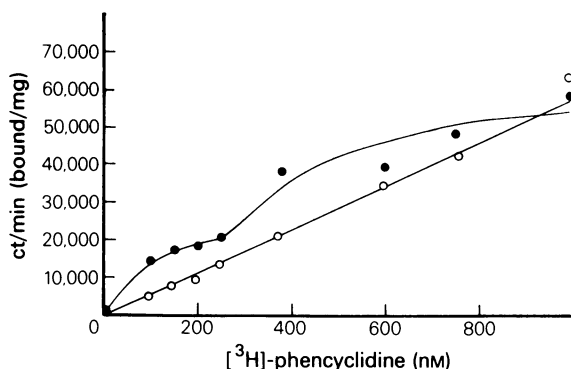
Figure 1 Binding of [³H]-phencyclidine ([³H]-PCP) to guinea pig ileum longitudinal muscle-myenteric plexus homogenates as a function of increasing concentration of radioactive ligand. Aliquots of homogenate (1 ml, 0.5 mg protein) in buffer were incubated in triplicate at 4°C for 30 min with various concentrations of [³H]-PCP. Specific binding (●), defined as total binding minus binding in the presence of non-radioactive phencyclidine (100 μM), as well as nonspecific binding (○) is shown. One of three experiments.

binding minus the binding in the presence of 100 μM PCP and was found to be saturable with respect to radiolabelled ligand concentration (Figure 1). Half-maximal binding occurred at approximately 36 nM [³H]-PCP. Linear regression analysis indicated that at 100 μM [³H]-PCP, specific binding represented 73% of total binding. In contrast, non-specific binding (binding which occurred in the presence of 100 μM PCP) was not saturable and increased linearly with increasing [³H]-PCP (Figure 1). Scatchard analysis (Figure 2) indicated two classes of binding sites, one with an apparent dissociation constant, K_D, of 36 nM and one with a K_D of about 2 μM. These apparent dissociation constants are very dependent on salt concentration. Increasing the Tris concentra-

Table 1 Relative potencies of phencyclidine (PCP) and related drugs in displacement of [³H]-phencyclidine binding to guinea-pig ileum

Drug	IC_{50} (μM)	Relative potency
PCP	0.5	1.00
TCP	0.3	1.67
PCE	0.4	1.25
PHP	0.45	1.11
Ketamine	5.5	0.09
PCM	1.0	0.5
Naloxone	>100	<0.005

TCP = 1,1-(2-thienyl)cyclohexylpiperidine;
PCE = N-ethyl-1-phenylcyclohexylamine,
PHP = 1-(1-phenylcyclohexyl)pyrrolidine;
PCM = 1-(1-phenylcyclohexyl)morpholine.

Figure 2 Scatchard plot of the binding of [³H]-phencyclidine ([³H]-PCP) to guinea-pig ileum longitudinal muscle-myenteric plexus preparation in 5 mM Tris-Cl, pH 7.4. Aliquots of homogenate (1 ml, 0.5 mg protein) in buffer were incubated in triplicate at 4°C for 30 min with various concentrations of [³H]-PCP. Specific binding, defined as total binding minus binding in the presence of non-radioactive phencyclidine (100 μM) is shown. One of three experiments. The two components of the graph were fit by linear regression analysis.

tion from 5 to 50 mM caused approximately a 5 fold increase in the apparent K_D.

Competition for binding sites with phencyclidine derivatives and other drugs

Table 1 illustrates the relative potencies of various phencyclidine derivatives in displacement of radiolabelled PCP. The range of concentrations of the competing ligands was 0.1–10 μM. The most potent drugs were TCP and PCE; PCM was the least effective. It is interesting to note that naloxone did not significantly affect high affinity binding of [³H]-PCP even when present at a concentration 10,000 fold higher than the radiolabelled ligand.

Effects of phencyclidine and its derivatives on binding of radiolabelled opiates

Table 2 illustrates the effects of PCP and its derivatives on the high affinity binding of [³H]-DHM and [³H]-DALA. PCP (10 μM) did not significantly inhibit [³H]-DALA (2 nM) or [³H]-DHM (2 nM) binding to ileum membranes. TCP (5 μM) also had no effect on [³H]-DHM binding but had a weak effect on [³H]-DALA binding. In contrast, ketamine (8 μM) had a weak inhibitory effect on the binding of [³H]-DHM, but no significant effect on the binding of [³H]-DALA. Higher concentrations of PCP, TCP, and ketamine were able to produce a more substan-

Table 2   Displacement of stereospecific opiate binding of guinea-pig ileum by phencyclidine (PCP) and related drugs

Drug	Concentration (μM)	Stereospecific binding of radiolabelled ligand (% total binding)	
		[^{3}H]-DHM	[^{3}H]-DALA
TCP	5	105 ± 2	83 ± 4
	10	86 ± 7	75 ± 7
PCP	10	94 ± 7	103 ± 3.5
	25	80 ± 5	93 ± 17
Ketamine	8	81 ± 6	99 ± 1
	80	94 ± 12	46 ± 0.5

Results are the means ± s.e.mean from at least three experiments, each carried out in triplicate.
DHM = dihydromorphine; DALA = D-Ala^2-metenkephalinamide; TCP = 1-1-(2-thienyl)cyclohexylpiperidine.

tial inhibition of the radiolabelled narcotic binding to opiate receptors.

Effects of 1-1-(2-thienyl)cyclohexyl piperidine and phencyclidine on the response to transmural electrical stimulation at 0.1 Hz

The effects of TCP and PCP on transmural electrical stimulation (0.1 Hz) are illustrated in Figures 3a and b. Both TCP and PCP produced a concentration-dependent inhibition of contraction height. TCP was more potent than PCP; the ED$_{50}$ for TCP was 13 μM and the ED$_{50}$ for PCP was 19 μM. Both of these were considerably more potent than ketamine, the ED$_{50}$ of which was 80 μM.

Effects of naloxone and tolerance formation on response to 1-1-(2-thienyl)cyclohexylpiperidine and phencyclidine

Pretreatment with naloxone, partially attenuated inhibitory responses to all concentrations of TCP and PCP that were tested (Figures 3a and b). Similar results were found with ketamine where 8×10^{-5} M produced a 45.8 ± 3.3 s.e.mean% ($n = 4$) inhibition before and a 24.0 ± 4.0 s.e.mean% ($n = 3$) inhibition after pretreatment with naloxone. The percentage of the total inhibition that was sensitive to naloxone was greatest at the lower concentration and least at the highest concentration of either drug. Ilea that were taken from guinea-pigs that were chronically exposed to morphine for 3 days by subcutaneous pellet implantation were at least 10 times less sensitive to the inhibitory effects of morphine (ED$_{50}$ of 2.5×10^{-7} M in naïve preparations compared with an ED$_{50}$ of 2.5×10^{-6} M in tolerant-dependent preparations). In these preparations neither the TCP- nor the PCP-induced inhibition of contraction height was affected by pretreatment with naloxone (10^{-7}–10^{-6} M). Thus, the naloxone-reversible com-

ponent of the TCP- or PCP-induced inhibition was not evident.
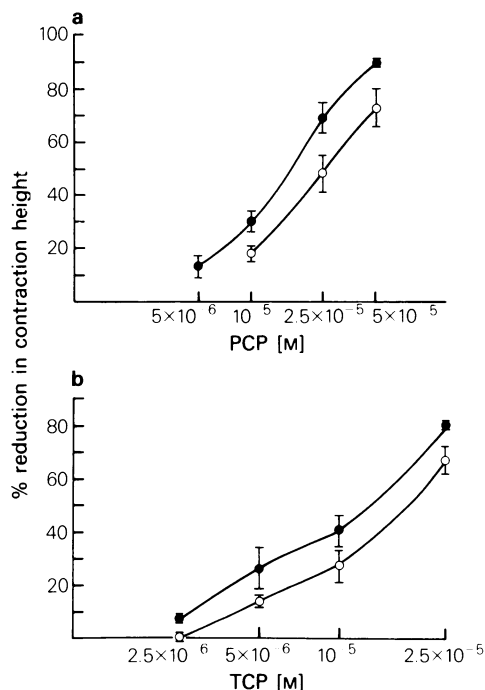
Figure 3   Effects of naloxone on the concentration-response curve for phencyclidine (PCP) and 1-1-(2-thienyl)cyclohexylpiperidine (TCP) to inhibit contractions produced in response to 0.1 Hz electrical stimulation. Concentration-response curves were constructed for PCP (a) and TCP (b) before (●) and after (○) preincubation (3 min) with naloxone (1 μM). Each point is the mean of 4 separate experiments (12 determinations for each concentration); vertical lines show s.e.mean.

Effects of phencyclidine pretreatment on dose-response curves to acetylcholine

In order to evaluate the anti-cholinoceptor properties of PCP in the gut, concentration-response curves of the resting ileum to ACh were constructed. Figure 4 illustrates that, although PCP $(2.5 \times 10^{-5}\,M)$ antagonized excitatory responses to exogenous ACh $(2 \times 10^{-8}–10^{-6}\,M)$, it caused a shift to the right in the concentration-response curve to ACh that was not parallel with the control concentration-response curves (4 separate experiments). Thus, PCP does not interact with the muscarinic cholinoceptor in a strictly atropine-like competitive fashion. Pretreatment with PCP did not result in a significant inhibition of the noncholinergic contracture produced by KCl $(5–25\,\text{mM};\ n=3,\ P>0.5)$ or substance P $(4 \times 10^{-11}–4 \times 10^{-10}\,M;\ n=3,\ P>0.5)$. In these experiments excitatory responses to KCl and substance P were determined in the presence of atropine $(2\,\mu M)$ to eliminate the component of the contracture due to the release of endogenous ACh.

Discussion

The present study indicates that the ileum of the guinea-pig contains binding sites for PCP and its derivatives that appear similar to those previously found in brain homogenates with respect to specificity, relative affinity of various ligands and sensitivity to peptidases and salt concentration. The LM-MP preparation manifests specific, saturable binding of $[^3H]$-PCP that is distinct from opiate receptor sites. These sites are sensitive to heat, trypsin and salt concentration as are those found in brain (Zukin & Zukin, 1979; Vincent, Vignon, Kartalovski & Lazduński, 1980a). Analysis of the binding by the method of Scatchard reveals two binding sites with K_D values of 36 nM and 2 μM. The K_D of the higher affinity site is very similar to the K_D described for the binding in brain (Vincent *et al.*, 1979; Zukin & Zukin, 1979). Moreover the rank order of potency of PCP derivatives to displace $[^3H]$-PCP very closely parallels the rank order obtained *in vitro* in the rotorod test (Vincent *et al.*, 1979; Zukin & Zukin, 1981).

Maayani & Weinstein (1980) suggested that the filtration method might be unsuitable for detecting the pharmacologically relevant binding sites of $[^3H]$-PCP. However, more recent work (Vincent *et al.*, 1980a; Vincent, Vignon, Kartalovski & Lazdunski, 1980b) suggests solutions to those problems and shows that appropriate use of the rapid filtration technique should permit the detection of the pharmacologically relevant PCP binding sites.

One effect of occupying the ileal PCP binding site
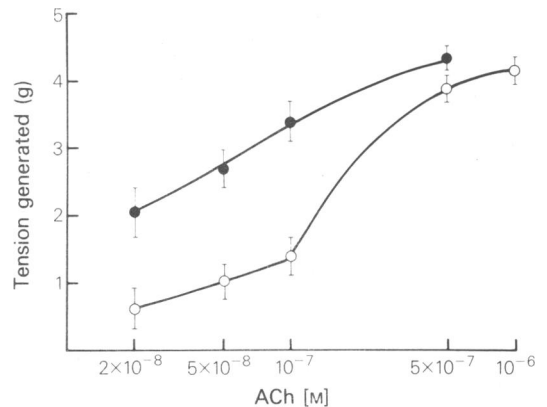
Figure 4   Effects of pretreatment with phencyclidine (PCP) on response of intact segments of guinea-pig ileum to exogenous acetylcholine (ACh). Log concentration-response curves to ACh were determined before (●) and after (○) a 2 min incubation with PCP (25 μM). Each of the indicated concentrations of ACh were administered once every 4 min until the magnitudes of at least 3 successive contractures were within 5% of each other. The ACh was left in contact with the tissue until a maximal contracture was obtained. The mean magnitude of these responses was used to construct the control concentration-response curve to ACh (●); vertical lines show s.e.mean. Following construction of the control concentration-response curve, the gut was pretreated with PCP (25 μM) for 2 min after which it was challenged with the indicated concentrations of ACh. Each point representing the magnitude of contracture to ACh following PCP pretreatment is the mean magnitude of response (±s.e.mean) of at least 4 separate determinations as described above for the control concentration-response curve. After construction of the concentration-response curve in the presence of PCP, the preparation was washed extensively and contractures to ACh were again determined in the absence of PCP. For each concentration shown, responses after washout of PCP were within 5% of the original response. The curves shown form one of 4 complete concentration-response determinations generated using ilea from 4 different animals. In each case, the concentration-response curve to ACh, in the presence of PCP, showed an inflection at 0.1 μM ACh.

is an inhibition of the contractile response of the ileum to electrical stimulation (0.1 Hz). Interestingly, the rank order of potency for this effect (TCP > PCP > ketamine) is identical to the rank order for displacing $[^3H]$-PCP from binding sites in LM-MP (Table 1) and brain (Vincent *et al.*, 1979) homogenates as well as their rank order of potency established in the rotorod test (Vincent *et al.*, 1979; Zukin & Zukin, 1979).

Analysis of the pharmacological effects of phencyclidines on the guinea-pig ileum suggests that these drugs can interact with the enteric cholinergic and

enkephalinergic systems. The inhibition produced by phencyclidines on the response to 0.1 Hz stimulation was partially reversed by naloxone. Moreover, the naloxone-reversible component was abolished in the ileum made tolerant to morphine. This observation indicates that at least a portion of the inhibition of response to 0.1 Hz electrical stimulation is mediated via endogenous opioid systems that are known to be present in the enteric nervous system. It is difficult to determine if the interaction between phencyclidines and the enteric opioid system(s) is due only to their ability to bind to opiate receptors or if an indirect effect via the release of endogenous opioids is also involved. Certainly, in concentrations in excess of 5 μM for TCP and 25 μM for PCP, these drugs can clearly bind to the opiate receptor and so direct effects are obviously involved. This binding to opiate receptors has also been observed in brain homogenate (Vincent et al., 1978). However, concentrations of TCP (5 μM) and PCP (25 μM) that were unable to produce appreciable displacement of [^{3}H]-DHM binding (Table 2) were nevertheless still able to produce a naloxone-reversible inhibition of 0.1 Hz induced contractions. Dihydromorphine is the prototypic ligand for the putative μ receptor, the opiate receptor subtype proposed to modulate release of acetylcholine in the guinea-pig ileum (Lord, Waterfield, Hughes & Kosterlitz, 1977). Thus, at least part of the naloxone-reversible component of PCP- and TCP-induced inhibition at low concentrations could be mediated indirectly via the release of an endogenous opioid. Alternatively PCP and TCP might exert their biological effect of inhibiting transmitter release at a receptor occupancy that is so low that it is not detected in displacment studies, i.e., PCP and TCP might have a high efficacy at opiate receptors despite a rather low affinity.

In addition to interacting with endogenous opioid systems, PCP-like drugs can also antagonize the response of the gut smooth muscle to exogenous acetylcholine (Figure 4). The anti-acetylcholine properties of phencyclidines in the CNS are well documented (Maayani & Weinstein, 1979). It should be noted, however, that PCP does not produce a parallel shift to the right in the dose-response curve to acetylcholine as would be expected if it were acting as a pure competitive antagonist of the muscarinic receptor like atropine. It should be noted that pretreatment with PCP (25 μM) did not produce a decrease in the overall responsiveness of the gut smooth muscle. The magnitude of the noncholinergic contractures produced by KCl (5–25 mM) and substance P (4×10^{-11}–4×10^{-10} M) were not significantly decreased. Thus, a specific interaction between PCP and some component of the system that subserves muscle responses to ACh must be considered. This result suggests that at least some of the PCP binding sites demonstrated in homogenates of LM-MP might be found in association with, or actually be a component of the muscarinic cholinoceptor complex. This idea would be consistent with the findings of Albuquerque, Tsai, Aronstan, Witkop, Eldefrawai & Eldefrawi (1980) who have shown that PCP can affect potassium channels associated with the cholinoceptor.

The ability of PCP to interact with both enteric neurones as well as gut smooth muscle is somewhat analogous to the actions of noradrenaline which also has a presynaptic inhibitory effect on the release of ACh and a direct inhibitory effect on the gut smooth muscle. Moreover, the inhibitory effect of PCP on the response of the gut smooth muscle to ACh could be considered somewhat analogous to the postsynaptic actions of opiates that have been described in the spinal cord (Zieglgansberger & Bayerl, 1976).

The further establishment of the guinea-pig ileum preparation as a model for studying the actions of phencyclidines could significantly enhance the search for effective antidotes to PCP intoxication (such as specific phencyclidine antagonists), as well as accelerate our understanding of the neuronal mechanisms that subserve its effects on the CNS.

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