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Phencyclidine ("angel dust") analogs and σ opiate benzomorphans cause cerebral arterial spasm

(vascular smooth muscle/basilar artery/middle cerebral artery/psychotomimetic activity/κ opiates)

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ABSTRACT **Several psychotomimetic phencyclidine (PCP) analogs—*N*-ethyl-1-phenylcyclohexylamine (PCE), *N*-[1-(2-thienyl)cyclohexyl]piperidine (TCP), *N*-[1-(thienyl)cyclohexyl]pyrrolidine (THP), ketamine, and *N,N*-dimethyl-1-phenylcyclohexylamine (PCDEA)—were tested on basilar and middle cerebral arteries of the dog *in vitro* and found to induce contraction in these blood vessels with a maximal contractile activity (i.e., intrinsic activity) similar to that of PCP. The concentration–effect curves of these compounds were found to be parallel to the curve of PCP ($P < 0.01$). The relative potency was PCE > TCP > PCP > THP > PCDEA > ketamine. A PCP analog with no psychotomimetic activity, 1-piperidinocyclohexanecarbonitrile (PCC), did not induce the blood vessels to contract, nor did the opiate morphine. Three psychotomimetic benzomorphans—pentazocine, cyclazocine, and *N*-allylnorcyclazocine—were found to: (*i*) also produce contraction; and (*ii*) have concentration–effect curves parallel to the curve of PCP, but with reduced intrinsic activities (i.e., maximal tensions were lowered) compared to PCP. A κ opiate, ethylketocyclazocine, relaxed the blood vessels in a dose-dependent manner. This study provides direct evidence for a distinct PCP receptor on cerebral blood vessels and suggests that certain benzomorphans may produce cerebral vasospasm via PCP–receptor interactions.**

In a previous paper, Altura and Altura suggested that specific biologically active phencyclidine (PCP; "angel dust") receptors might be located on cerebral blood vessels (1). In that paper, it was reported that PCP produced cerebral arterial spasms that could not be antagonized by some known amine antagonists or by an inhibitor of prostaglandin synthesis. To explore further the possibility that such receptors subserving contraction exist in these blood vessels, we obtained a number of synthetic analogs of PCP with known and different binding affinities to the PCP receptors in rat or guinea pig brain (2–5), and with various degrees of behavioral activity as determined in rat rotarod (rotating rod) and discrimination tests (2–7). Several of these analogs [e.g., *N*-ethyl-1-phenylcyclohexylamine (PCE) and *N*-[1-(2-thienyl)cyclohexyl]piperidine (TCP)] are already being used illicitly among street drug users (8, 9).

We now report similar orders of potencies in binding of the agents to rat brain receptors, behavioral activity, and affinity of the drugs for the cerebral blood vessel receptor. In addition, it was found that some benzomorphans with behavioral activity that appears to be mediated through σ receptors also caused the blood vessels to contract and had affinities to the PCP receptor in cerebral arteries similar to the affinities of some of the PCP analogs. These benzomorphans, however, displayed greatly reduced maximal contractile activity (intrinsic activity). Additionally, a typical κ opiate-receptor binder was found to produce, rather surprisingly, dose-dependent relaxation of cerebral vessels.

METHODS

Mongrel dogs of either sex weighing 15–20 kg were anesthetized with sodium pentobarbital (30 mg/kg). After craniotomy the brain was rapidly removed and the basilar and middle cerebral arteries were excised (10). Helical strips were cut from segments of these cerebral arteries; the strips were 15 mm long by 1.5–2.0 mm wide (10). The strips were suspended isometrically under a 1-g (9.8-millinewton) tension and incubated in a 10-ml muscle chamber containing normal Krebs–Ringer bicarbonate solution (composition in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 10; and $NaHCO_3$, 25) at 37°C, through which O_2 (95%)/CO_2 (5%) was bubbled (10). The force of contraction was measured with a Grass FT-03 force–displacement transducer and recorded on a Grass model 7 polygraph. Two hours after incubation under tension, the preparations were tested with 30 mM KCl, then after a 0.5-hr washout, followed by relaxation, complete dose–response curves of the various agents were determined. These curves were always compared to a PCP dose–response curve, determined either before or after the curve for the analog, benzomorphan, etc., because there was some variability of the strips with time.

Threshold (i.e., minimal effective concentrations) and EC_{50}s (concentration needed to develop 50% of the maximal responses to each agonist) were calculated in order to be able to compare the relative affinity of the analogs and other compounds (1). Maximal contractile responses were utilized as a measure of intrinsic activity (11). We tested for parallelism of the dose–response curves as described in ref. 12. In selected experiments, we incubated cerebral arteries with various pharmacologic antagonists (i.e., atropine sulfate, methysergide maleate, phentolamine, or naloxone, each at 0.5 μg/ml) in order to determine whether the benzomorphans might act via cholinergic, serotoninergic, α-adrenergic, or μ opiate receptors. The means (±SEM) of the thresholds, EC_{50}s, and maximal contractile tensions were calculated, and when appropriate were compared for statistical significance of differences by means of Student's *t* test.

RESULTS

All agents tested except for 1-piperidinocyclohexanecarbonitrile (PCC), morphine, and ethylketocyclazocine (EKC) produced various degrees of contraction on both types of cerebral

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Abbreviations: PCP, phencyclidine; PCE, *N*-ethyl-1-phenylcyclohexylamine; TCP, *N*-[1-(2-thienyl)cyclohexyl]piperidine; THP, *N*-[1-(thienyl)cyclohexyl]pyrrolidine; PCDEA, *N,N*-dimethyl-1-phenylcyclohexylamine; PCC, 1-piperidinocyclohexanecarbonitrile; EKC, ethylketocyclazocine.

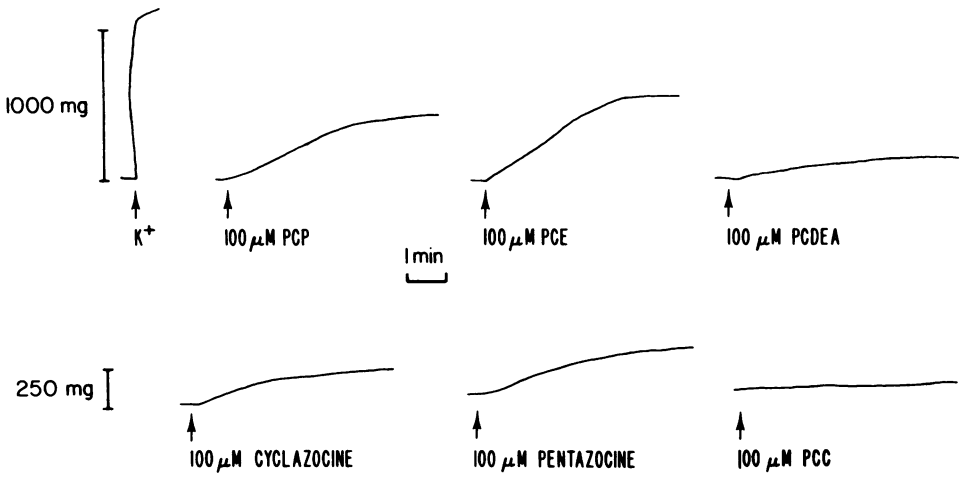

FIG. 1. Comparative contractile responses of an isolated canine middle cerebral artery to addition of 30 mM potassium (K^+), and 100 μM doses of PCP, PCE, *N,N*-dimethyl-1-phenylcyclohexylamine (PCDEA), cyclazocine, pentazocine, and PCC.

blood vessels (Fig. 1). Morphine and PCC failed to produce any response up to a concentration of 5 mM. As can be seen in Figs. 2 and 3 (and Table 1), the order of potency (based on EC_{50}s) of the PCP analogs was PCE > TCP > PCP > THP > PCDEA > ketamine. This order of potency was true for both the middle cerebral and basilar arteries. All the concentration–effect curves for analogs of PCP that induced the blood vessels to contract proved to be parallel to the PCP concentration–effect curves (e.g., Fig. 4; $p < 0.01$).

The psychotomimetic benzomorphans, classed as σ opiates, also induced these cerebral blood vessels to contract (Figs. 2 and 3; Table 1). Their order of potency was pentazocine > cyclazocine > *N*-allylnorcyclazocine. The concentration–effect curves of these molecules, like those for the PCP analogs, were either parallel to the concentration–effect curve of PCP or occupied the same space (e.g., Fig. 5). Whereas the intrinsic activities of the PCP analogs were not significantly different from one another ($P > 0.05$), those for the benzomorphans were drastically and significantly reduced ($P < 0.01$): in the middle cerebral artery the maximum induced by pentazocine was reduced by 38%, that by cyclazocine by 50%, and that by *N*-allylnorcyclazocine by 70%, as compared to the PCP maximal contraction. With the basilar artery, the maximum induced by pentazocine was reduced by 25%, that by cyclazocine by 36%, and that by *N*-allylnorcyclazocine by 60%, as compared to the PCP maximal contraction.

The typical κ opiate agonist EKC, in the concentration range 42–800 μM, was found to relax baseline tension and, in a dose-dependent manner, to relax blood vessels that had been induced to contract by prostaglandin $F_{2\alpha}$.

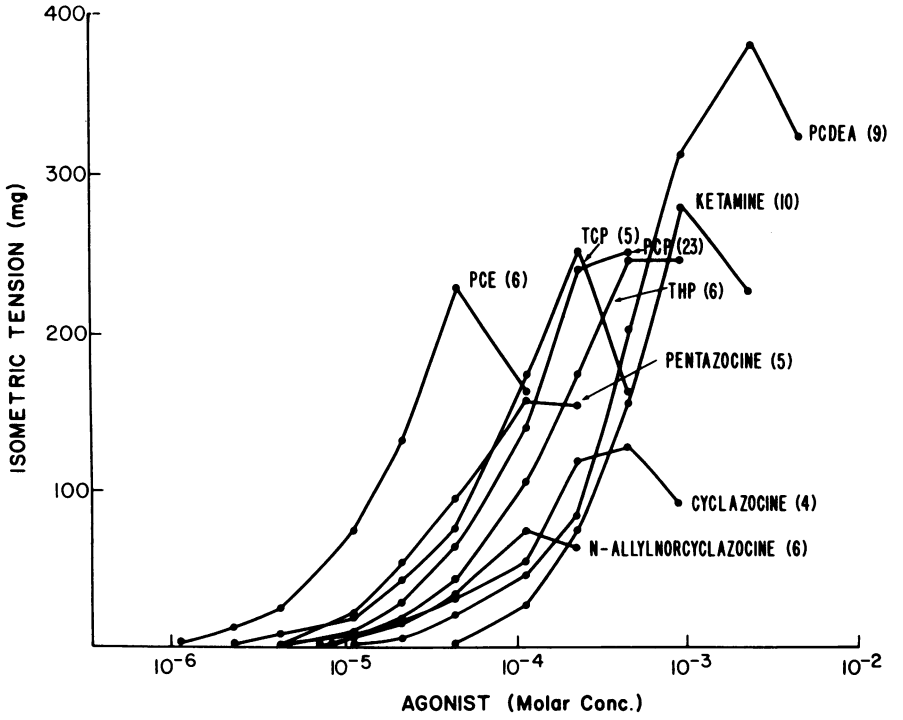

FIG. 2. Comparative contractile potencies of PCP, PCP analogs, and benzomorphans on isolated canine middle cerebral arteries. Numbers in parentheses indicate the number of different animals utilized. Each point represents the mean value for each concentration of agonist utilized. SEMs for threshold concentrations, EC_{50} concentrations, and maximal tensions can be found in Table 1. THP, *N*-[1-(thienyl)cyclohexyl]pyrrolidine; *N*-allylnorcyclazocine is also known as SKF-10,047.

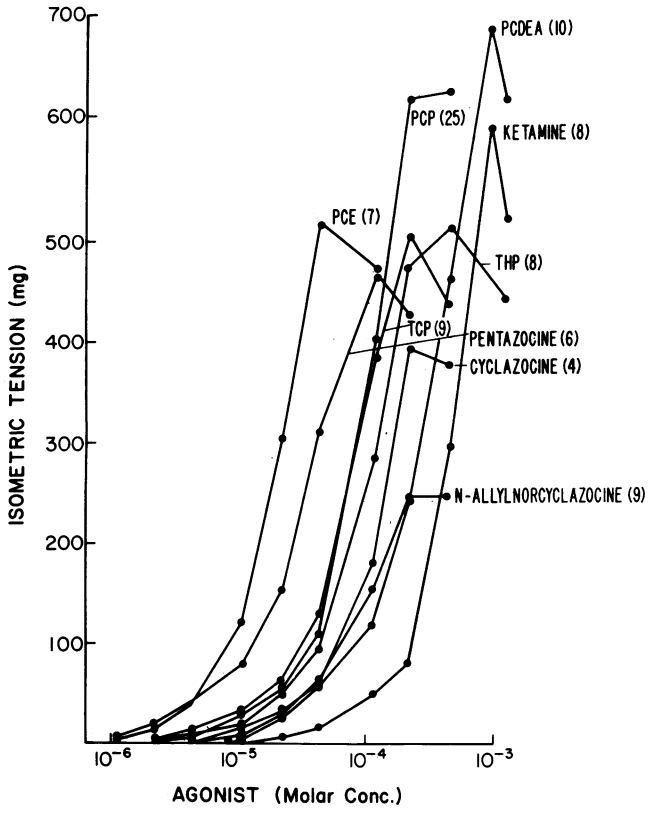

FIG. 3. Comparative contractile potencies of PCP, PCP analogs, and benzomorphans on isolated canine basilar arteries. Symbols and notations are similar to those of Fig. 2.

When single doses of the PCP analogs as well as the σ opiate benzomorphans were compared, a similar contractile pattern was always obtained—i.e., a gradual slow contraction rising steadily for low, effective concentrations, and a faster upsurge followed by a fall-off at the higher concentration (e.g., Fig. 1).

Neither atropine, methysergide, phentolamine, nor naloxone was found to inhibit or attenuate the contractile responses induced by benzomorphans or PCP ($n = 4$).

DISCUSSION

This report demonstrates a contractile activity on cerebral blood vessels for a number of PCP analogs that display behavioral activity *in vivo*, lending support to our previous suggestion of the presence of a specific receptor that subserves contraction in response to PCP in the cerebral vasculature (1). The present findings show a great similarity to those of Quirion *et al.* (5) and others (2–4), that the binding of these agents to rat or guinea pig brain correlated closely to their ability to elicit behavioral activity. Here, those agents that bound to the brain slices most potently also elicited minimal (or threshold) contraction in the lowest concentrations; the order of potency of contraction following closely the order of displacement of PCP in the rat brain slices. Also, as in the binding studies, which showed that the μ opiate ligand morphine does not interact with the PCP binding site (2, 3, 5), the psychotomimetic σ opiate benzomorphans do seem to interact with the same receptor as PCP, because they caused contractions in similar concentrations and with similar patterns. But, more importantly, the concentration–effect curves of these molecules, like those for the PCP analogs, were all parallel to the concentration–effect curve for PCP on both types of cerebral blood vessels studied. In addition, preliminary experiments ($n = 4$) indicate that incubation of cerebral arteries with supramaximal concentrations of cyclazocine prevents, completely, any subsequent contractile response to PCP. This seems to lend support to the concept "that the σ opiate effects to the benzomorphan opiates are due to their fortuitous ability to interact with the PCP receptor" (5).

With regard to the intrinsic activity of the σ opiate benzomorphans on cerebral arteries, however, it is apparent that these molecules do not have the ability to bring about complete activation of the PCP receptor in cerebrovascular smooth muscle, even though they are extremely potent in terms of their EC_{50}s (Table 1). The decreased maximal contractile effects of the benzomorphans are probably due to the fact that they possess κ opiate receptor activity, and therefore, their net intrinsic activities constitute a summation of a partial relaxation and a partial contraction. In addition, it may be that the racemic mixtures used here may have different activity on cerebral blood vessels than on cortical areas in the brain. In this context, it has recently been demonstrated that stereoisomers of *N*-allylnorcyclazocine act differently with reference to behavioral actions in both rats and monkeys in comparison to PCP (13).

The results we obtained with the typical κ opiate agonist EKC, relaxation of baseline tension and of agonist-induced contraction, were unexpected and may be of considerable interest. A recent study by Quirion *et al.* (14) shows that this com-

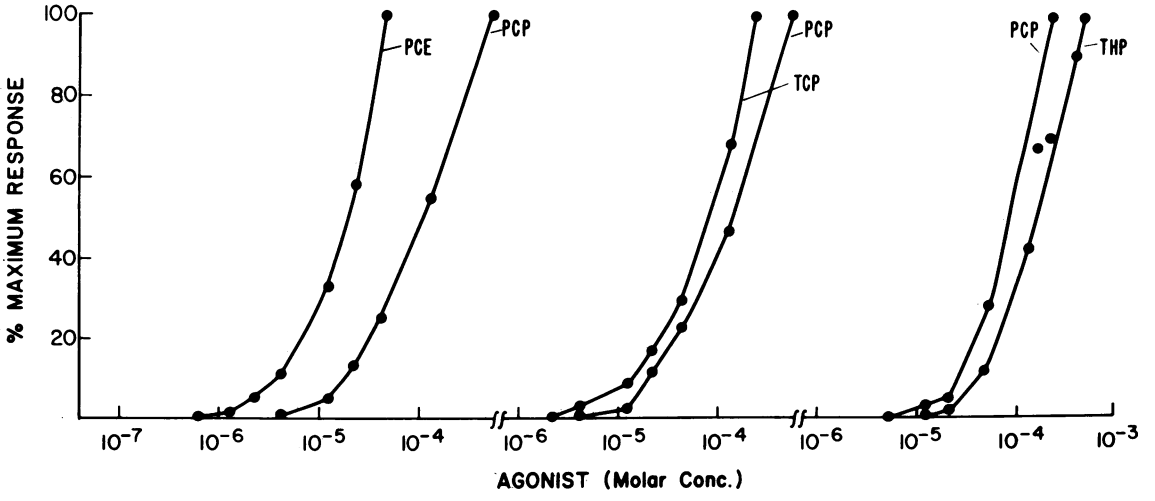

FIG. 4. Contractile concentration–effect curves for PCP analogs parallel PCP concentration–effect curves in isolated canine middle cerebral arteries ($P < 0.01$).

Table 1. Relative contractile sensitivities of canine middle cerebral arteries and basilar arteries to PCP, PCP analogs, and opiates

Drug	*n*	Minimal effective concentration, μM	EC_{50}, μM	Maximal tension, mg	Relative potency in inducing contraction*	Relative potency in PCP rat olfactory bulb binding assay†
			Middle cerebral arteries			
PCP and analogs						
PCP	23	18.2 ± 1.1	92 ± 0.6	252 ± 14.5	1.0	1.0
PCE	6	6.92 ± 2.24	19 ± 3.2	229 ± 43.5	4.8	6.0
TCP	5	11 ± 3.1	75 ± 14.7	270 ± 25.5	1.2	1.7
THP	6	25 ± 9.7	150 ± 8	250 ± 22.6	0.6	0.8
PCDEA	9	68 ± 16.3	380 ± 20	383 ± 99.0	0.24	0.3
Ketamine	10	120 ± 18	500 ± 25	281 ± 11.8	0.18	0.1
PCC	6	0	0	0	0	<0.001
Opiates						
Pentazocine	5	16 ± 2.2	42 ± 12.5	157 ± 30.4	2.2	0.01
Cyclazocine	4	25 ± 6.5	150 ± 10	127 ± 40.2	0.6	0.8
N-Allylnorcyclazocine	6	41 ± 16.3	52 ± 4.4	75 ± 11.2	1.76	0.3
Morphine	4	0	0	0	0	0
			Basilar arteries			
PCP and analogs						
PCP	25	13.5 ± 2.8	82 ± 0.6	619 ± 21.7	1.0	1.0
PCE	7	5.11 ± 1.77	19 ± 0.1	517 ± 73.1	4.3	6.0
TCP	9	13.2 ± 4.3	62 ± 0.1	503 ± 43.3	1.3	1.7
THP	8	24.2 ± 5.6	90 ± 8.1	510 ± 70.1	0.9	0.8
PCDEA	10	42 ± 14.2	320 ± 19	682 ± 73.7	0.26	0.3
Ketamine	8	61 ± 15.9	400 ± 63	589 ± 62.8	0.2	0.1
PCC	6	0	0	0	0	<0.001
Opiates						
Pentazocine	6	3.0 ± 0.53	38 ± 11.4	462 ± 63	2.2	0.01
Cyclazocine	4	12 ± 0.1	125 ± 25	394 ± 11.9	0.66	0.8
N-Allylnorcyclazocine	9	35 ± 13.6	90 ± 8.0	246 ± 41.9	0.9	0.3
Morphine	4	0	0	0	0	0

* Based on EC_{50}s.
† Taken from data of Quirion *et al.* (5).

pound binds to a distinct class of opiate binding sites in rat brain slide-mounted sections, different from PCP, σ, μ, or δ opiate receptors.

In view of the present findings, one must consider the possibility that cerebrovascular smooth muscle may provide a useful tool to analyze the molecular constitution of the PCP receptor, and in addition it may provide a specific bioassay for PCP-like materials.

Last, the present findings seem to reinforce the idea suggested previously that some of the intoxicating and behavioral effects of PCP may be due to the action of this hallucinogen on the blood vessels of the brain (1).

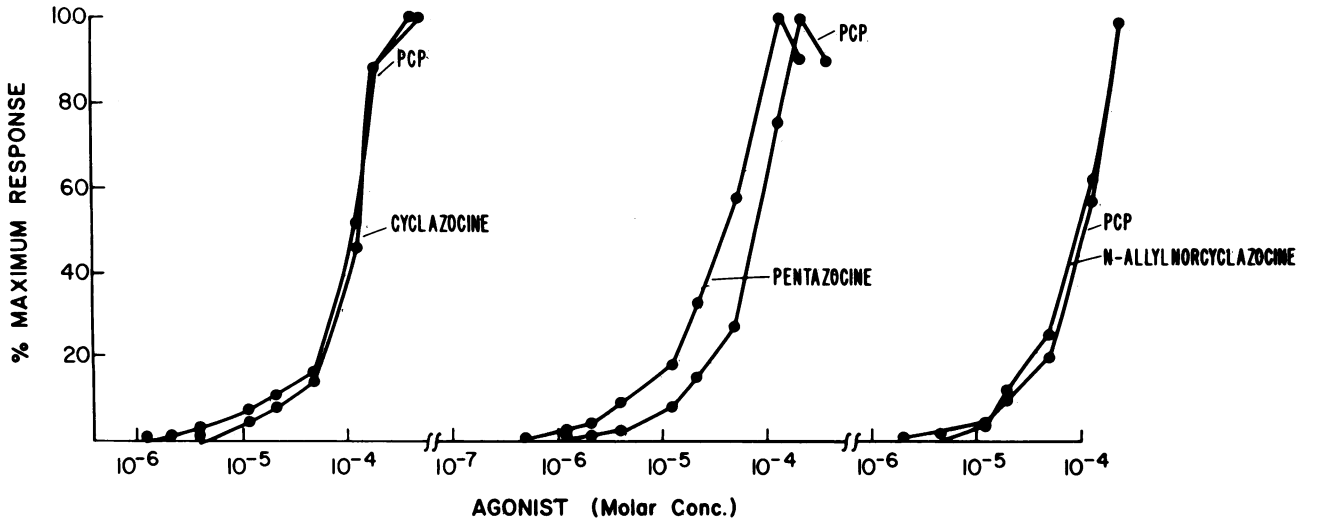

FIG. 5. Contractile concentration–effect curves for benzomorphans acting on isolated canine basilar arteries either parallel PCP concentration–effect curves (e.g., pentazocine) or occupy the same space as PCP concentration–effect curves (e.g., cyclazocine, *N*-allylnorcyclazocine) ($P < 0.01$).

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