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2-Amino-6-trifluoromethoxy benzothiazole (PK 26124), a proposed antagonist of excitatory amino acid neurotransmission, does not produce phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys.

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SUMMARY

PK 26124, a proposed excitatory amino acid antagonist, was compared to mephenesin and phencyclidine (PCP). In pigeons, PK 26124 and mephenesin produced loss of righting that was to some extent associated with eye closure and muscle relaxation, whereas PCP produced catalepsy, i.e., loss of righting without eye closure and without muscle relaxation. PK 26124, but not mephenesin, produced PCP-like discriminative stimulus effects in some but not all pigeons. In rats, PK 26124 and mephenesin produced loss of righting but did not induce locomotion, sniffing, swaying and falling, unlike PCP. In rhesus monkeys, PK 26124 did not induce ketamine-like discriminative stimulus effects. While PK 26124 may share some biochemical properties with excitatory amino acid antagonists these do not lead to behavioral effects similar to PCP.

Key words: phencyclidine, rhesus monkeys, pigeons, rats, drug discrimination, catalepsy, PK 26124, 2-amino-6-trifluoromethoxy benzothiazole, mephenesin, midazolam.

Certain behavioral effects of phencyclidine (PCP) may result from a PCP receptor-mediated reduction of neurotransmission at excitatory synapses utilizing N-methyl-D-aspartate (NMDA)-preferring receptors. Recently, we reported behavioral evidence in support of this hypothesis: 2-amino-5-phosphonovalerate (AP5), a potent and highly selective NMDA antagonist, produced PCP-like behavioral effects in pigeons and in rats (Koek, Woods, and Ornstein, 1986; Koek, Woods, Jacobson, and Rice, 1987a; Koek, Woods, and Ornstein, 1987b), and may produce PCP-like behavioral effects in rhesus monkeys (Woods, Koek, and Ornstein, 1987).

NMDA antagonists, and PCP-like drugs, are anticonvulsants, and, recently, 2-amino-6-trifluoromethoxy benzothiazole (PK 26124) has been reported to possess anticonvulsant properties (Mizoule, Meldrum, Mazadier, Croucher, Ollat, Uzan, Legrand, Gueremy, and Le Fur, 1985). The anticonvulsant properties of PK 26124 may be mediated by antagonism of excitatory amino acid neurotransmission (Benavides, Camelin, Mitrani, Flammand, Uzan, Legrand, Gueremy, and Le Fur, 1985).

The present study was aimed at characterizing the possible PCP-like behavioral effects of PK 26124. The ability of PK 26124 to induce PCP-like catalepsy and discriminative stimulus effects in pigeons, PCP-like directly observable effects in rats, and ketamine-like discriminative stimulus effects in rhesus monkeys was evaluated. PK 26124 is a benzazole, i.e., a compound with a benzene ring fused to a five-membered ring system with two or more heteroatoms, one of which is nitrogen. Certain other benzazoles have pharmacological properties (e.g., anticonvulsant, muscle relaxant) which resemble those of the centrally acting muscle relaxant, mephenesin (Domino, Unna, and Kerwin, 1952). Therefore, a second aim of the present study was to characterize the effects of mephenesin in the aforementioned behavioral procedures and to compare these with the effects of PK 26124.

METHODS

Subjects. Twenty-seven White Carneaux pigeons (Palmetto, Sumter, SC) were housed individually with water and grit freely available: 20 pigeons had continuous access to Purina pigeon checkers; 7 pigeons were maintained at 80% of their free-feeding weight by providing food in the home cage after each experimental session. Twenty male albino rats of the Sprague-Dawley strain (Harlan Industries, Indianapolis, IN) weighing 150-200 g, were housed individually and were given free access to food and water. Three rhesus monkeys were housed individually and were maintained at 80% of their free-feeding weight by providing Purina monkey chow in the home cage after each experimental session.

Apparatus. Apparatus was the same for the parallel experiments described in Koek, Woods, Rice, Jacobson, Huguenin, and Burke (1984; Koek et al., 1986; 1987a,b) and in Woods et al. (1987).

PCP-like catalepsy in pigeons. This procedure has been described in detail elsewhere (Koek et al., 1984). During an experimental session, drug injections were given i.m. at 20 min intervals and effects (i.e., presence or absence of loss of righting, head-drop, and eye closure) were assessed 15 min after each injection. Cumulative dosing continued until a dose was reached at which all animals showed catalepsy, or a limitation of further testing (e.g., solubility of drug) was imposed.

PCP-like discriminative stimulus effects in pigeons. Seven food-deprived pigeons were trained to discriminate between an i.m. injection of PCP (1 mg/kg; later in the experiment, the dose was lowered) and of saline.

PK 26124 produced PCP-key selection in some, but not all, of the pigeons for which the training dose remained at 1 mg/kg (n=3), indicating that PK 26124 might be a low-potency PCP agonist. If this partial generalization is indeed based on partial agonist properties of PK 26124, then 1) PK 26124 should at least partially antagonize the discriminative stimulus effects of 1 mg/kg of PCP, because the combination of a full agonist with a partial agonist results in competitive dualism (Ariens, Simonis and Van Rossum, 1964), and 2) PK 26124 should produce a higher proportion of PCP-key selections in the pigeons for which the training dose was lowered to 0.32 mg/kg of PCP (n=4), because a lowering of the training dose decreases the level of agonist activity that is required to induce drug-appropriate responding. Thus, PK 26124 was tested as a pretreatment to a training dose of 1 mg/kg of PCP, and when given alone to animals trained to discriminate between 0.32 mg/kg of PCP and saline.

PCP-like directly observable effects in rats. Rats were observed sequentially for 30 sec and were scored for a number of behaviors (see Koek et al., 1987b, Table 1), tested for the occurrence of loss of righting and anesthesia, injected, and immediately returned to the observation cage. Injections were given i.p. at 15-min intervals and behavioral effects were assessed 13-15 min after each injection by an observer who had no knowledge of the treatment received by the rats. Each drug was tested in a cumulative dosing procedure using four to eight rats per drug.

Ketamine-like discriminative stimulus effects in rhesus monkeys. The procedure has been described elsewhere (Woods et al., 1987). Training sessions consisted of a series of discrete trials, each preceded by a 10-min blackout period that was initiated by a s.c. injection of either saline of 1.8 mg/kg of ketamine.

During test sessions, all trials of the session were preceded by an injection of saline, except the second trial, which was preceded by an injection of a particular dose of the test compound. During each trial of a test session, 100 consecutive responses on either lever produced food. For each trial, the lever on which 100 consecutive responses accumulated first was defined as the selected lever; the rate of responding was measured by dividing the total number of responses on both levers by the duration of the trial (in sec). Test sessions were not conducted until the monkey produced more than 90% injection-appropriate responses in every trial of two consecutive training sessions.

Drugs. The compounds used were 2-amino-6-trifluoromethoxy benzothiazole (PK 26124; generously provided by Dr. R. Michaud, Pharmuka Laboratories, Gennevilliers, France), 3-[o-tolyloxy]-1,2-propanediol (mephenesin; Sigma Chemical Co., St. Louis, MO), phencyclidine and ketamine hydrochloride (Warner-Lambert/Parke-Davis and Co., Ann Arbor, MI) and midazolam (Hoffmann-La Roche, Nutley, NJ). PK 26124 was dissolved in lactic acid to which sterile water was added. Mephenesin was suspended in sterile water along with Tween 80. All other drugs were dissolved in sterile water. Doses of PCP are expressed as the free base; doses of all other drugs are expressed in the forms described above.

RESULTS

PCP-like catalepsy in pigeons. The results obtained with PK 26124, mephenesin and midazolam (Fig. 1) indicated that there was no dose level at which all subjects show a loss of righting without the concomitant occurrence of head-drop and/or eye closure (the dose of PK 26124 was increased until the solubility limit was reached). Therefore, none of these drugs produced PCP-like catalepsy as defined herein (i.e., loss of righting without head-drop and without eye closure). It should be noted, however, that PK 26124- and mephenesin-induced loss of righting was associated to a lesser degree with head-drop and/or eye closure than the loss of righting induced by midazolam. Thus, in this procedure, PK 26124 and mephenesin produced effects that were neither like PCP nor like midazolam, but were intermediate in terms of the amount of head-drop and/or eye closure that was induced.

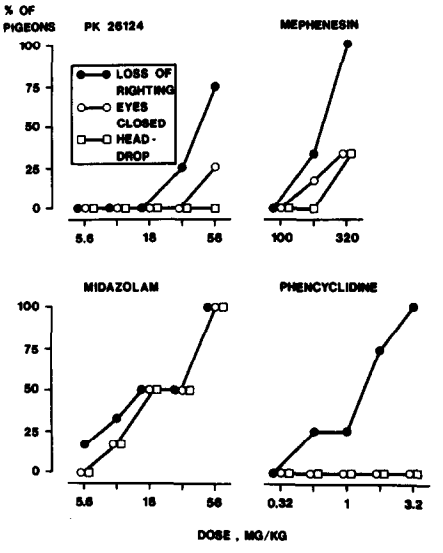

Fig. 1. Dose-effect curves of drug-induced loss of righting, eye closure and head-drop in pigeons (n=4-6). Ordinates: percentage of subjects showing loss of righting, eye closure, head-drop; abscissae: cumulative i.m. dose administered (milligrams per kilogram).

PCP-like discriminative stimulus effects in pigeons. At each training dose (i.e., 1.0 and 0.32 mg/kg), PCP exerted dose-dependent effects on the percentage of PCP-key selections (Fig. 2). Note that the potency of PCP to induce PCP-key selection increased at least three-fold by lowering the training dose from 1.0 to 0.32 mg/kg. PCP did not significantly affect the mean key selection latency, at the training doses used herein ($F[4,12]=1.9$, $P>0.10$).

PK 26124 induced PCP-appropriate responding in some, but not all pigeons that were trained to discriminate 1.0 mg/kg of PCP. However, pretreatment with varying doses of PK 26124 prior to administration of 1.0 mg/kg of PCP failed to attenuate the discriminative stimulus effects of this training dose of PCP. Further, the percentage of PCP-key selections produced by PK 26124 was lower, instead of higher, in pigeons that were trained with 0.32 mg/kg of PCP than in pigeons that were trained with 1.0 mg/kg of PCP. In contrast to PK 26124 and PCP, mephenesin induced exclusively selection of the saline key, both in the high and in the low training dose group. In a dose range of 3.2 to 10 mg/kg, PK 26124 did not significantly affect the mean key selection latency, neither when given alone, nor when given in combination with 1.0 mg/kg PCP ($F[3,6]=1.73$, $P<0.20$; $F[4,12]=1.86$, $P<0.10$); however, all pigeons failed to select either key after the administration of 18 mg/kg of PK 26124. Mephenesin (32 mg/kg) significantly increased the mean key selection latency in the high training dose group ($F[3,6]=17.63$, $P<0.005$), but not in the low training dose group ($F[3,9]=1.25$, $P>0.20$); at 56 mg/kg, two animals in the high training dose group and one animal in the low training dose group failed to select either key during the 1-hr session.

PCP-like directly observable effects in rats. Rats receiving multiple saline injections during an experimental session did not show any of the behaviors listed in Table 1 of Koek et al. (1987b); the data obtained in this group served as control values in testing the statistical significance of drug effects. PCP significantly induced, at increasing doses, locomotion (3.2 mg/kg), sniffing (5.6 mg/kg), swaying (5.6 mg/kg), falling (10 mg/kg) and Straub tail (56 mg/kg). Mephenesin significantly induced locomotor activity (100 mg/kg) and loss of righting (560 mg/kg), but not sniffing, swaying, falling and Straub tail; PK 26124 induced loss of righting (18 mg/kg) only. Two of the four rats tested with 56 mg/kg of PK 26124 and one of the four rats tested with 560 mg/kg of mephenesin died.

Ketamine-like discriminative stimulus effects in rhesus monkeys. The training dose of ketamine (1.8 mg/kg) induced full drug-appropriate responding at 15 min and at 30 min after its administration, that was followed by a gradual return to full saline-appropriate responding. The rate of responding was decreased only at 15 min after the administration of ketamine. PK 26124 produced, however, neither ketamine-

appropriate responding nor suppression of the overall rate of responding. The solubility limit of PK 26124 was reached at the highest dose tested (5.6 mg/kg).

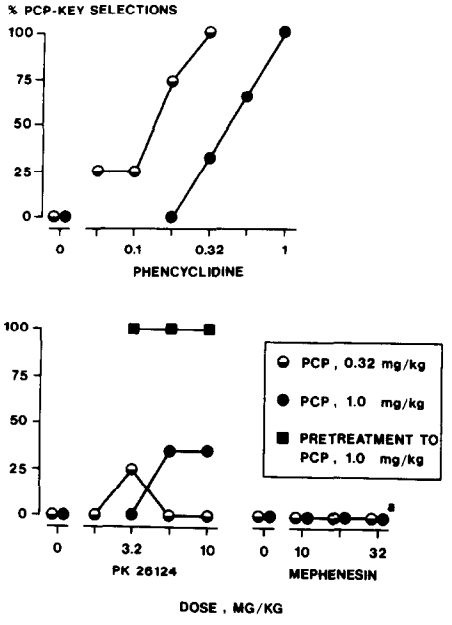

Fig. 2. Discriminative stimulus effects of PCP, PK 26124 and mephenesin in pigeons (n=3) trained to discriminate 1 mg/kg of PCP from saline, and in pigeons (n=4) trained to discriminate 0.32 mg/kg of PCP from saline. PK 26124 was not only tested when given alone, but also when given 10 min prior to the administration of the 1 mg/kg training dose of PCP. Data points that are marked with an "a" were obtained at doses that significantly increased the mean key-selection latency. Ordinates: percentage of PCP-key selections; abscissae: i.m. dose administered (milligrams per kilogram). Dose-response curves of PCP are replotted from other sources (Koek et al., 1987a).

DISCUSSION

Recently we have shown that NMDA antagonists produce PCP-like behavioral effects (Koek et al., 1986; 1987a, b; Woods et al., 1987). The present study was aimed at exploring the possible PCP-like behavioral effects of PK 26124, a proposed antagonist of excitatory amino acids (Benavides et al., 1985). Further, the behavioral effects of the benzazole PK 26124 were compared with the effects of the muscle relaxant mephenesin, because some benzazoles have mephenesin-like activity (e.g., Domino et al., 1952).

In pigeons, PK 26124 and mephenesin produced loss of righting that was associated with eye closure, and failed to induce PCP-like discriminative stimulus effects in all subjects; in rats both drugs produced loss of righting, but failed to induce sniffing, swaying and falling. The similarity between the behavioral effects of PK 26124 and mephenesin is in agreement with and extends the finding that certain benzazoles have mephenesin-like pharmacological properties.

Neither PK 26124 nor mephenesin produced PCP-like catalepsy in PCP-like discriminative stimulus properties in pigeons and PCP-like directly observable effects in rats, unlike the NMDA antagonist, AP5 (Koek et al., 1987a,b). Further, the partial generalization produced by PK 26124 in PCP-discriminating pigeons does not seem to be based on partial PCP agonist properties of PK 26124, because PK 26124 neither antagonized partially the stimulus effects of PCP, nor produced more drug-appropriate responding as the training dose of PCP was lowered. Finally, PK 26124 did not produce ketamine-like discriminative stimulus effects in rhesus monkeys. Together, these findings would be inconsistent with the hypothesis that certain effects of PCP-like drugs may be mediated by a reduction of excitatory amino acid neurotransmission at NMDA-preferring receptors, if PK 26124 is indeed a selective antagonist of excitatory amino acids. In the study of Benavides et al. (1985) two models were used to study the effects of PK 26124 on excitatory amino acid neurotransmission: 1) excitatory amino acid-induced increases in cerebellar content of cyclic guanosine monophosphate, and 2) NMDA- and electrically-evoked acetylcholine release from striatum; because of the antagonist actions of PK 26124 in both models, PK 26124 was

proposed as an antagonist of excitatory amino acid neurotransmission. Recently, however, the effects of PK 26124 have been compared to other reference standards using procedures that may more clearly distinguish NMDA antagonists from local anesthetics (Tsai, Steel, McPherson, Taylor, Wood and Lehmann, 1987). Because the effects of PK 26124 on NMDA- and KCl-evoked striatal acetylcholine release, on cerebellar cGMP content and on aconitine-induced inhibition of tritiated L-glutamate uptake were like the effects of the local anesthetic lidocaine and were unlike the effects of the NMDA antagonist, 2-amino-7-phosphonoheptanoate (AP7), and the PCP-like drug, tiletamine, Tsai et al. (1987) have suggested that PK 26124 is not a selective antagonist at NMDA-type receptors. The absence of PCP-like behavioral activity of PK 26124 observed herein is consistent with this suggestion. Whether lidocaine would show the same pattern of anticonvulsant activity as PK 26124 has not been determined.

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