Br. J. Pharmac. (1983), **79**, 219–223

Sedative effects of PK 9084 and PK 8165, alone and in combination with chlordiazepoxide

Sandra E. File

Department of Pharmacology, The School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX

1 The sedative effects in rats of two phenylquinolines, PK 9084 and PK 8165 (5–50 mg/kg), were examined in a holeboard: both when given alone and when given in conjunction with chlordiazepoxide (5 mg/kg).

2 Both phenylquinolines produced significant dose-related decreases in locomotor activity and rearing, with an ED_{50} about twice that for chlordiazepoxide.

3 When the phenylquinolines were combined with chlordiazepoxide the degree of sedation was equal to that seen with either drug given alone, whichever produced the greater sedation; the sedative effects of the two drugs were never additive.

4 PK 9084 (10 and 50 mg kg) significantly reduced rectal temperature, as did chlordiazepoxide (5 mg/kg), but there was no addition nor interaction of their effects.

5 Both phenylquinolines also reduced exploratory head-dipping, as did chlordiazepoxide, but in combination they antagonized each other's effects.

6 The classification of the phenylquinolines as non-sedative anxiolytics, acting as agonists at the benzodiazepine receptors needs revision.

Introduction

Two phenylquinolines, PK 9084 and PK 8165, that potently displace benzodiazepines from their binding sites have been claimed to be non-sedative anxiolytics (Le Fur, Mizoule, Burgevin, Ferris, Heaulme, Gauthier, Gueremy & Uzan, 1981). However, the evidence for their anxiolytic action, at doses similar to the ED_{50} for chlordiazepoxide (4.5 mg/kg), was obtained from a conflict test in rats; whereas mice were used to show that the ED_{50} for sedation was about ten times higher. The comparison of doses between different species is always difficult, but is further complicated in this case because the mice used by Le Fur *et al.* (1981) were relatively insensitive to the sedative actions of benzodiazepines: the ED_{50} for chlordiazepoxide was 25 mg/kg, which is ten times higher than the sedative dose in rats (File, 1982). Furthermore, since the ED_{50} for sedation caused by the quinolines was only about twice that for chlordiazepoxide, this suggests that the former may not differ so markedly from the benzodiazepines in producing sedation.

It was therefore the purpose of the present experiment to examine further any possible sedative effects of PK 9084 and PK 8165, both when they were administered alone and when they were given together with chlordiazepoxide. Three doses of the quinolines were chosen, covering the range previously found to be behaviourally active (Le Fur *et al.*, 1981), and 5 mg/kg chlordiazepoxide was chosen since this is the lowest dose to produce sedation consistently in our rats (File, 1982). The rats were tested in a holeboard, which permits independent measurement of locomotor activity, rearing and directed exploration (measured by head-dipping).

Methods

Animals

Male hooded Lister rats (Olac Ltd., Bicester), approximately 200 g, were housed in groups of six with food and water freely available and in a 13 h light: 11 h dark cycle, with lights on at 06 h 00 min.

Drugs

PK 9084 hydrochloride (2-phenyl-4-[2-(3-piperidinyl) ethyl]-quinoline) and PK 8165 hydrochloride (2-phenyl-4-[2-(4-piperidinyl) ethyl]-

© The Macmillan Press Ltd 1983

quinoline) (Pharmuka) and chlordiazepoxide hydrochloride (Roche Products Ltd) were dissolved in water to give injection volumes of 2 ml/kg. All doses are expressed as the hydrochloride salt.

Apparatus

The holeboard was a wooden box (60 × 60 × 35 cm) with 4 equally spaced holes (3.8 cm in diameter) in the floor. Infrared cells under each hole provided an automated measure of the number of head-dips made and the time spent head-dipping (measures of directed exploration; File & Wardill, 1975). Infrared cells in the walls of the box (4.5 and 11 cm above the floor) provided automated measures of locomotor activity and rearing, respectively. The illuminance on the floor of the box was 33 scotopic lux.

Procedure

The experiment was conducted over two weeks. In one week, 80 rats were randomly allocated ten each to the following groups: control (water); chlordiazepoxide (CDP 5 mg/kg); PK 9084 (5, 10 or 50 mg/kg); CDP (5 mg/kg) plus PK 9084 (5, 10 or 50 mg/kg). In the other week, 80 rats were allocated ten each to similar groups but with PK 8165. Each day 20 rats were tested between 08 h 00 min and 11 h 00 min in an order randomized for drug treatment.

All rats received two i.p. injections: either water, PK 9084 or PK 8165 1 h before test; and then either water or CDP 30 min before test. Each rat was placed singly in the holeboard for a 7.5 min trial, at the end of which any boluses were removed and the box was thoroughly wiped. For the rats tested with PK 9084 their rectal temperatures were measured immediately after the holeboard trial.

Statistics

The data from each measure in the holeboard were analysed by a two-way analysis of variance in which the quinoline (PK 9084 or PK 8165) and CDP were the two factors. The quinoline × CDP interactions were then examined in more detail by an analysis of the simple main effects. Comparisons of individual groups with the control group were made with Dunnett's test.

Results

Figure 1 shows that both PK 9084 and PK 8165 produced dose-related decreases in motor activity ($F(3,72) = 29.68$ and 30.99, respectively; $P < 0.0001$). For both compounds, the first dose to differ significantly from the controls was 10 mg/kg ($P < 0.05$). For some doses (5 and 10 mg/kg PK 9084, and 5 mg/kg PK 8165) the combination with CDP resulted in motor activity scores significantly ($P < 0.01$) lower than those seen with the same doses of quinolines given alone. However, the motor activity scores in the combined groups were never lower than those seen either in the CDP alone group (for the 5 and 10 mg/kg phenylquinoline doses), or that seen in the quinoline alone groups (for the 50 mg/kg groups), see Figure 1. In other words, the

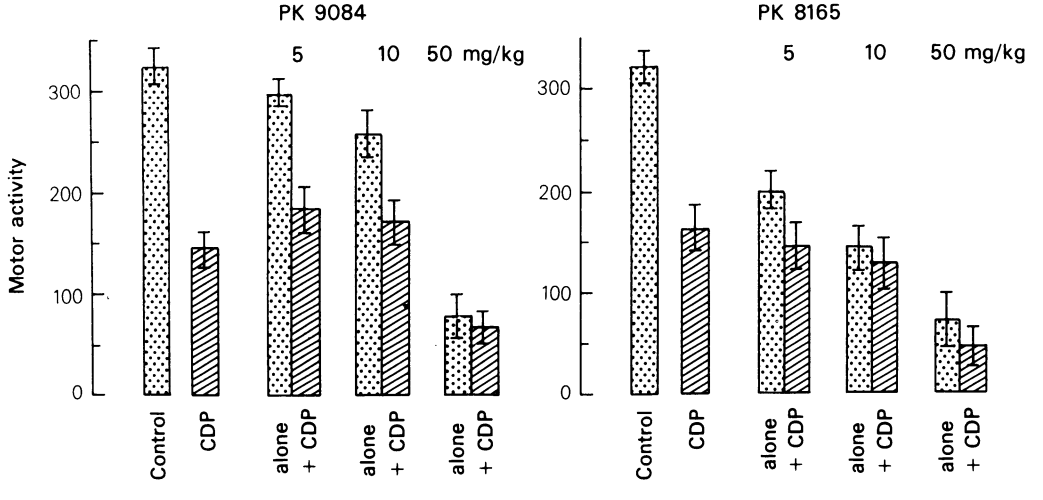

Figure 1 Mean motor activity scores for controls; for rats injected with 5 mg/kg chlordiazepoxide (CDP) and for rats injected with PK 9084 and PK 8165 (5–50 mg/kg) alone, and in combination with 5 mg/kg CDP. Vertical lines indicate s.e.mean.

Table 1 Mean (±s.e.mean) number of rears and number of head-dips made by rats treated with PK 9084 or PK 8165 alone (5–50 mg/kg) or in combination with chlordiazepoxide (CDP, 5 mg/kg) during a 7.5 min holeboard test

PK dose (mg/kg)	5	10	50
(a) *Number of rears*			
PK 9084 alone	103.8 ± 4.63	89.6 ± 9.86	25.4 ± 6.98
PK 9084 + CDP	62.7 ± 6.07	51.0 ± 9.37	21.0 ± 4.86
PK 8165 alone	83.9 ± 8.10	58.9 ± 9.29	21.1 ± 6.80
PK 8165 + CDP	54.6 ± 9.15	46.9 ± 6.84	15.7 ± 4.75
(b) *Number of head-dips*			
PK 9084 alone	19.0 ± 2.44	15.9 ± 2.09	2.6 ± 1.07
PK 9084 + CDP	12.9 ± 1.41	15.5 ± 2.90	5.0 ± 1.71
PK 8165 alone	14.8 ± 2.38	7.9 ± 1.53	3.0 ± 1.44
PK 8165 + CDP	10.5 ± 1.94	9.8 ± 2.02	3.3 ± 1.34

Vehicle-injected control rats had a mean (s.e.mean) number of rears = 113.7 ± 2.64 and number of head-dips 21.6 ± 1.77. Rats injected with CDP alone (5 mg kg) had a mean number of rears 54.2 ± 7.03 and number of head-dips 10.9 ± 1.61.

sedative effects of CDP and the phenylquinolines were never additive.

As can be seen from Table 1 the pattern of results shown by the number of rears was almost identical to that seen with motor activity. Both PK 9084 and PK 8165 significantly ($F(3,72) = 34.79$ and 32.59, respectively; $P < 0.0001$) reduced rearing when given alone, and this was significant from the dose of 10 mg/kg upwards ($P < 0.025$). CDP alone significantly reduced rearing ($P < 0.0001$); the combination of CDP with certain doses (PK 9084 at 5 and 10 mg/kg; PK 8165 at 5 mg/kg) resulted in lower scores than were seen for the quinolines alone.

Table 1 also shows the mean number of head-dips made by rats injected with water or with various doses of the two phenylquinolines, alone or in combination with CDP. Both PK 9084 and PK 8165 produced significant dose-related decreases in the number of head-dips made ($F(3,72) = 19.65$ and 18.15, respectively; $P < 0.001$), as did CDP when given alone ($P < 0.001$). However, when CDP was combined with the quinolines there was no reduction compared with the same dose of quinoline when given alone.

Figure 2 shows that a similar pattern of results emerged for the time spent head-dipping. Both PK 9084 and PK 8165 significantly reduced the time spent head-dipping ($F(3,72) = 8.87$ and 12.19,

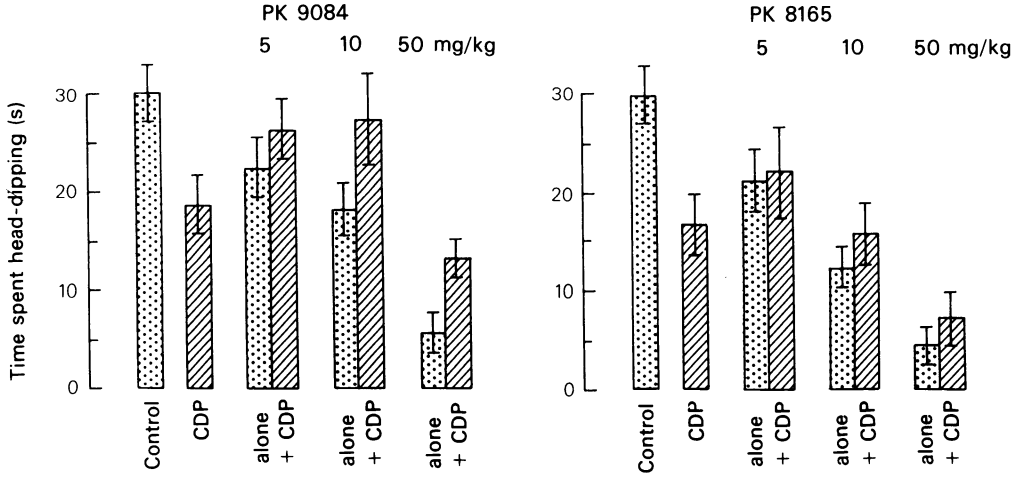

Figure 2 Mean time spent head-dipping (s) by controls; by rats injected with 5 mg/kg chlordiazepoxide (CDP) and for rats injected with PK 9084 and PK 8165 (5–50 mg/kg) alone, and in combination with 5 mg/kg CDP. Vertical lines indicate s.e.mean.

Table 2 Mean (±s.e.mean) rectal temperatures (°C) immediately after the holeboard test for rats treated with vehicle (water) or PK 9084 (5, 10 or 50 mg/kg) alone or in conjunction with chlordiazepoxide (CDP, 5 mg/kg)

PK 9084 (mg/kg)	0	5	10	50
Alone	38.1 ± 0.09	37.9 ± 0.26	37.2 ± 0.24	33.9 ± 0.48
+ CDP	36.8 ± 0.23	37.1 ± 0.28	36.6 ± 0.26	33.9 ± 0.55

$P<0.001$) when given alone. CDP when given alone also reduced the time spent head-dipping ($P<0.001$). However, when CDP and the quinolines were combined there was no longer any reduction below control levels for PK 9084 (5 and 10 mg/kg) and no reduction for PK 8165 below the level produced by injection of the quinoline alone.

Table 2 shows the mean (±s.e.mean) body temperature of the rats immediately after the holeboard test. Both PK 9084 and CDP significantly reduced rectal temperature ($F(3,72) = 51.57$, $P<0.001$; and $F(1,72) = 8.20$, $P<0.01$, respectively), but there was no addition or interaction of their effects. The drop in body temperature after PK 9084 alone was significant from the dose of 10 mg/kg ($P<0.01$).

The top dose (50 mg/kg) of both quinolines proved toxic and 5 days after this dose the rats died; a few of the rats that received 10 mg/kg also died 5–15 days after the dose. In all cases, post-mortem revealed liver damage.

Discussion

The toxicity of the top dose (50 mg/kg) of the quinolines means that the scores of the rats given this dose must be interpreted with caution. The low activity and exploration scores could have been secondary to sickness. However, it is unlikely that the sedation seen with the much lower doses is due to sickness since only a few of the rats receiving 10 mg/kg doses died, and this was from liver damage which took several days to develop. Also, the motor activity scores for the rats given quinolines plus chlordiazepoxide were lower than the scores for those given the quinolines alone, whereas there was no evidence for increased toxicity in the combined treatment groups. Although non-specific causes of sedation can never be totally excluded, the different patterns of interactions with CDP that were reflected in motor activity, compared with head-dipping, would be difficult to account for on this basis.

CDP (5 mg/kg) reduced motor activity and rears by approximately 50%, and a roughly equivalent degree of sedation was found with 10 mg/kg of the phenylquinolines. This confirms the results from mice (LE Fur *et al.*, 1981) that the ED_{50} for sedation caused by the phenylquinolines is twice that found for chlordiazepoxide. For both CDP and PK 9084 the dose-response curve for hypothermia was very similar to that seen for motor activity and therefore the possibility that sedation was secondary to the drop in body temperature cannot be excluded. When the quinolines were given in conjunction with CDP the degree of sedation was equal to that seen with either drug given alone, whichever produced the greater sedation. This pattern of results suggests that CDP and the quinolines were not acting at the same receptor to produce sedation.

The effects of the combination of chlordiazepoxide and PK 9084 on the time spent head-dipping is of particular interest. When given on its own, CDP significantly reduced head-dipping, as did the phenylquinolines. Yet when the two drugs were combined, rather than producing an additive sedative effect, CDP was able to counteract the effects of PK 9084 (5 and 10 mg/kg) so that the scores did not differ from the controls. A similar pattern of results has been found with the benzodiazepine antagonist, CGS 8216, a pyrazoloqinoline (Czernik, Petrack, Kalinsky, Psychoyos, Cash, Tsai, Rinehart, Granat, Lovell, Brundish, & Wade, 1982). Like CDP, when given alone CGS 8216 reduces head-dipping; but when the two drugs are combined, there is mutual antagonism of their effects (File & Lister, 1983a). The classification of benzodiazepine 'agonists' and 'antagonists', on the basis of binding parameters, does not seem to apply to their effects on exploratory head-dipping.

The discovery of a non-sedative anxiolytic would clearly be of clinical value, as well as being invaluable in the search for the neural basis of anxiolytic and sedative actions of drugs acting at benzodiazepine receptors. However, to be of use it is essential that any such claim is based on rigorous experimental evidence. The evidence that PK 8165 and PK 9084 are anxiolytics is not strong. It comes from only one conflict experiment (Le Fur *et al.*, 1981) and a partial anxiolytic effect for PK 9084 in the social interaction test (File & Lister, 1983b). The original claim that the phenylquinolines were not sedative is clearly not true for rats; and, in their interactions with chlor-

diazepoxide on head-dipping, the phenylquinolines resembled the benzodiazepine antagonist, CGS 8216. In summary, it seems that the classification of PK 8165 and PK 9084 as non-sedative anxiolytics, acting as agonists at one class of benzodiazepine receptors (Le Fur *et al.*, 1981) needs revision.

S.E.F. is a Wellcome Trust Senior Lecturer. I am grateful to Pharmuka for the gift of PK 9084 and PK 8165, and to Peter Mabbutt for expert technical assistance.

References

CZERNIK, A.J., PETRACK, B., KALINSKY, H.J., PSYCHYOS, S., CASH, W.D., TSAI, C., RINEHART, R.K., GRANAT, F.R., LOVELL, R.A., BRUNDISH, D.E. & WADE, R. (1982). CGS 8216: receptor binding characteristics of a potent benzodiazepine antagonist. *Life Sci.*, **30,** 363–372.

FILE, S.E. (1982). Behavioural pharmacology of the benzodiazepines. Paper read to Eastern CINP group, Hong Kong. *Prog. Neuropharmac.*, (in press).

FILE, S.E. & LISTER, R.G. (1983a). RO 15–1788 and chlordiazepoxide each reverse the reduction in exploration caused by CGS 8216. *Br. J. Pharmac.*, (in press).

FILE, S.E. & LISTER, R.G. (1983b). Quinolines and enxiety: anxiogenic effects of CGS 8216 and partial anxiolytic profile of PK 9084. *Pharmac. Biochem. Behav.*, (in press).

FILE, S.E. & WARDILL, A.G. (1975). Validity of head-dipping as a measure of exploration in a modified holeboard. *Psychopharmacologia*, **44,** 53–57.

LE FUR, G., MIZOULE, J., BURGEVIN, M.C., FERRIS, O., HEAULME, M., GAUTHIER, A., GUEREMY, C. & UZAN, A. (1981). Multiple benzodiazepine receptors: evidence of a dissociation between anticonflict and anticonvulsant properties by PK 8165 and PK 9084 (two quinoline derivatives). *Life Sci.*, **28,** 1439–1448.

(*Received November 23, 1982. Revised January 6, 1983.*)