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Interactions Between Phencyclidine and Central Nervous System Depressants Evaluated in Mice and Rats[1]

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WESSINGER, W. D. AND R. L. BALSTER. *Interactions between phencyclidine and central nervous system depressants evaluated in mice and rats.* PHARMACOL BIOCHEM BEHAV 27(2) 323–332, 1987.—The effects of phencyclidine (PCP) alone and in combination with the CNS depressants, pentobarbital (PB) or ethanol (ETOH), were determined in mice using the inverted screen test and in rats using disruption of milk drinking behavior. The effects of PB and ETOH alone, and in combination, were also determined so that the PCP combinations could be compared to this clinically relevant interaction. These homergic drug interactions were analyzed using the dose-addition model by isobolographic analyses. Most drug combinations resulted in shifts to the left of the dose-effect curves relative to the dose-effect curves for the drugs alone; in no cases were shifts to the right (antagonism) observed. In general, the interactions between PCP and ETOH or PB were quantitatively less (infra-additive) than the interaction between the CNS depressants (dose-additive) when studied in mice. In the rat studies, the interactions between PCP and ETOH or PB were, overall, quantitatively greater (dose-additive or supra-additive) than the ETOH-PB interactions (infra-additive). Since even infra-additive interactions may result in substantially enhanced effects, these results suggest that coabuse of PCP with CNS depressant drugs could produce marked behavioral toxicity.

Drug interactions Mice Rats Phencyclidine Pentobarbital Ethanol Isobolographic analysis Dose-addition

PHENCYCLIDINE (1-(1-phenylcyclohexyl)piperidine; PCP) is an arylcyclohexylamine with an unusual spectrum of pharmacological action. As a popular drug of abuse, PCP is undoubtedly often taken in conjunction with other abusable substances. The interactions of PCP with central nervous system (CNS) depressants is of particular concern as both drug classes share some similar depressant actions [5] and clinical and forensic reports suggest these drug combinations are popular [1, 2, 23]. PCP has been reported to enhance the depressant effects of CNS depressants such as the barbiturates or ethanol (ETOH) in rodents [9, 11, 14, 33, 34], pigeons [36], rhesus monkeys [14, 27, 43], patas monkeys [37] and humans [13]. Interestingly, studies in squirrel monkeys [14,15] did not show a similar enhancement and species differences may be involved, although other factors have not been ruled out.

With the exception of the studies by Brunet *et al.* [11] and Woolverton and Balster [43] the above mentioned studies employed the effect-addition model to evaluate the observed interaction effects. This model predicts that the combination of dose a and dose b of drugs A and B should produce a combined effect equal to the arithmetic sum of the individual effects. This is referred to as effect-additive and deviations from the predicted effects are described accordingly [18]. Strictly speaking, the effect-addition model is only appropriate for homergic drugs in which the dose-effect functions are linear and pass through the origin, a situation which would certainly be rare, or perhaps nonexistent when considering drug effects *in vivo*. When a threshold dose is necessary, or the dose-effect curves are sigmoid or otherwise non-linear, then combined effects are not simply arithmetically additive. Both dose and effect should be taken into account when predicting the effects of drug combinations [17]. An alternative approach for homergic drug combinations which takes both dose and effect into account is the dose-addition model. This model thus makes predictions

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about an interaction that relate the potencies of the interacting drugs. The working hypothesis of the dose-addition model is that the two interacting drugs act as if they were different forms of the same substance, differing only perhaps in potency. To test this hypothesis, the "dose" of the mixture needed to produce a selected response (often the 50% level of effect) is determined [29]. The dose-addition model provides three categories that specify the magnitude of leftward shifts of the dose-effect curves. If the leftward shift is equal to what would be expected if doses of drug B substitute for drug A in proportion to their relative potencies, the interaction is dose-additive. Alternatively, the shifts may be greater than expected, that is, the dose combination may produce greater than dose-additive effects which is termed supra-additive. In the case where the leftward shift is less than expected, the interaction would be described as infra-additive. Interpretation of the dose-addition model can be aided by the use of isobolographic analysis, first introduced by Loewe and Muishnek [26] and reviewed by Gessner [19]. Isobolographic methods facilitate data reduction and allow a graphic depiction of dose-addition analysis. Because the dose-addition model provides more information concerning the nature and magnitude of interactions and has a sounder theoretical basis, this model was chosen to describe the interactions reported herein. Interested readers are referred elsewhere for further discussion of the advantages and disadvantages of both models [17, 39, 40, 42].

The present experiments were conducted to further characterize the interactions of PCP with CNS depressants, specifically pentobarbital (PB) and ETOH. In addition, the interactions between PCP and these CNS depressants were quantitatively compared to the interactions between the two CNS depressants. Comparison of the interaction effects of combinations of PCP and CNS depressants with the effects of drug combinations of known clinical significance (PB and ETOH) permits an evaluation of relative risk of toxicity for persons coabusing such combinations. These studies were conducted in mice and rats using simple behavioral measures that exhibited monotonic, unidirectional drug effects. For these measures, PCP and PB, PCP and ETOH, and PB and ETOH were homergic drug pairs, that is, all three drugs produced similar effects.

METHOD

Animals

In the mouse experiment, the subjects were male, CD-1 mice (Charles River Laboratories, Wilmington, MA) which weighed 22–30 g at the time of the experiments. Upon arrival they weighed 22–24 g and were housed in groups of 10–15 per cage for 10–12 days prior to testing, with food (Rodent Laboratory Chow No. 5001, Ralston Purina Co., St. Louis, MO) and tap water continuously available. On the day before testing, the mice were moved to the test room and food deprived for 24 hr.

In the rat studies, different groups of 12 male Sprague-Dawley rats (Dominion Laboratories, Dublin, VA) were used to study each drug pair. The animals were earpunched for individual identification and allowed to gain weight to 300 g. During this time they were adapted to handling and trained to consume milk during daily 10-min access periods. Weights were maintained by adjusted post-session feeding (Rodent Laboratory Chow No. 5001) and tap water was continuously available. The rats were housed and tested in suspended stainless-steel, wire-mesh cages (18×19×25 cm).

Apparatus and Procedures

Drug effects in mice were measured using the inverted screen test as a measure of motor performance [4,16]. The screen test apparatus consisted of six wire mesh screens (13×13 cm) mounted horizontally on pedestals above a metal bar. A pivot on each end of the bar, located in the plane of the screens, allowed the screens to be rotated 180 degrees. The apparatus was mounted on ring stands, with the screens 60 cm above a table top. Six mice were placed on the individual screens and the screens were then rotated over a 1–2 sec period so that the mice were suspended from the bottom. The number of mice climbing to the top of the screens within 60 sec was recorded. Several hours prior to drug administration, the mice were tested for their ability to climb to the top of the screens. Only subjects which climbed to the top of the screen on one of two trials were used for testing purposes. Usually less than 5% of the subjects failed to meet this criterion. After pretesting, the mice were divided into groups of 6 per cage. All drugs were administered IP and the mice were tested 20 min after injection.

For the rat studies, the amount of milk consumed during a limited access period was used as the dependent measure [12]. Experimental sessions were usually conducted five days per week. For 10 min each day a solution of milk (2 parts tap water to 1 part Borden's "Eagle Brand" sweetened condensed milk) was placed on the cage front in a 50-ml plastic graduated-centrifuge tube fitted with a rubber stopper and drinking spout. At the end of the 10-min session, the amount of milk consumed (ml) by each subject was recorded. After adaptation to the drinking session procedures and weight stabilization, the rats were adapted to the injection procedure by administering 0.9% saline IP (1.0 ml/kg) 15 min prior to sessions on Tuesdays, Thursdays and Fridays for 5 sessions. Milk consumption was stable (less than 10% variation in the mean intake for three consecutive sessions) for each group prior to drug testing. During drug testing, the drugs or combinations were administered, IP, 15 min prior to the session on Tuesdays and Fridays. Vehicle (saline, 1.0 ml/kg) was administered on Thursdays and the amount of milk consumed in these sessions served as control.

Drug Effects

In both the mouse and the rat studies, the effects of PCP, PB and ETOH were examined alone. Combinations of PCP and PB, PCP and ETOH, and PB and ETOH were evaluated by administering the two drugs in two separate IP injections as closely timed as possible. For these interactions, the "fixed-dose method" [20] was employed. For example, in the mouse studies of the combination of PCP and PB, the dose-effect curves for PCP and PB alone were first determined. Then dose-effect curves for PB in combination with 0.5, 1.0 and 1.5 mg/kg PCP and for PCP in combination with 3.0 and 6.0 mg/kg PB were determined. The combinations of PCP and ETOH and PB and ETOH were similarly studied. Usually 12 naive mice were tested at each dose or dose combination and at least three doses were tested for each dose-effect curve.

For the rat studies, a within-subjects design was employed. A different group of 12 rats was used for determining the effects of each drug pair. For the first drug exposure, the lowest anticipated effective dose was administered, but these data were not used. This was done to eliminate possible novelty effects which might result in unreliable effects on first drug exposure. Then doses of one drug of the interaction

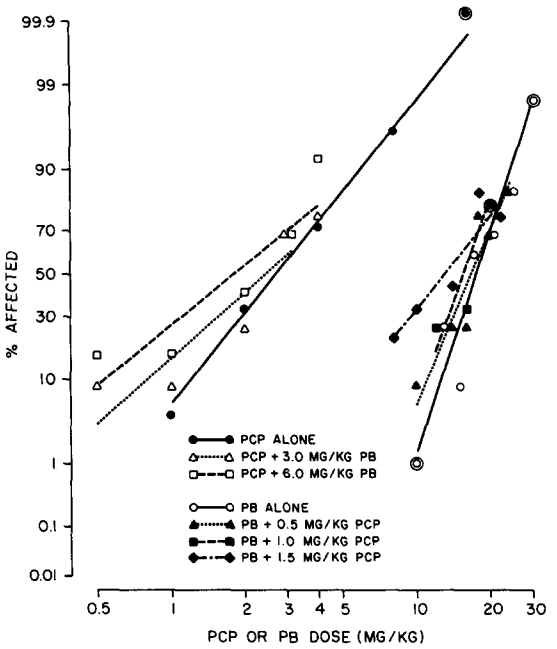

FIG. 1. Dose-effect functions for PCP, PB, and combinations tested with the inverted screen test for mice. Points are the observed effects of various doses or combinations; circled points are estimated effects for doses which resulted in 0 or 100% of the mice being unable to climb to the top of the screen; lines were fitted by a computer approximation [3] of the method of Bliss [7].

pair were administered in a mixed order to determine a dose-effect relationship for that drug alone. The same procedure was followed for the second drug of the drug pair. Secondly, the effects of drug combinations were determined by using a fixed dose of one of the drugs and combining it with various doses of the interacting drug in a mixed order until a dose-effect relationship could be determined. Following the testing of the combinations, the dose-effect curves for each drug alone were redetermined as before.

Drugs

PCP was prepared by diluting a stock solution of phencyclidine hydrochloride (Sernylan, Bioceutic Laboratories, St. Joseph, MO) or by dissolving phencyclidine hydrocloride (National Institute on Drug Abuse, Rockville, MD) with physiological saline to an appropriate concentration to give a 10.0 ml/kg injection volume for mice or a 1.0 ml/kg injection volume for rats. Doses are in terms of mg/kg of the salt. PB (sodium pentobarbital, USP, The Lannett Company, Philadelphia, PA) was dissolved in physiological saline to the appropriate concentration to give the same injection volumes. Doses are in terms of mg/kg of the salt and fresh solutions were made daily as needed. ETOH (95% ethanol, USP, Medical College of Virginia Pharmacy, Richmond, VA) was diluted to a 12.5% (v/v) solution containing 99 mg/ml of ethanol with physiological saline daily as needed [6,38]. This concentration was selected to avoid hemorrhagic lesions of the peritoneal membranes in the rat studies and to still be high enough to enable administration of the higher doses of ETOH tested [6]. Because of concentration-dependent effects on absorption, dose-like effects can be produced by simply varying the concentration of a fixed amount of ETOH [24]. In these experiments, the concentration was held constant and the volume injected varied to give the appropriate dose in terms of g/kg.

Data Analyses

In the mouse studies, the effects of each drug alone were determined twice during the course of the three series of drug interaction experiments. The data from the two determinations were combined for analysis. The nominal data obtained was analyzed by a computer approximation [3] of the method of Bliss [7] which provided the ED50's (dose which would be expected to cause 50% of the mice to be unable to climb to the top of the inverted screens within 60 sec) and 95% confidence limits. The ED50's for the combinations of fixed-doses of one drug with the interacting drugs were tested for significant differences ($p<0.05$) from the E50's of the interacting drug alone by using appropriate portions of Litchfield and Wilcoxon's analysis [25]. Similarly, the slope functions for each dose-effect curve were determined and tested for parallelism ($p<0.05$) [25].

For the rat studies, the effects of each drug alone of an interaction pair were determined before and after the testing of drug combinations. These data were combined for dose-effect analysis for each of the three groups of rats. Vehicle effects measured during each dose-effect determination were averaged to serve as control values. The portions of the dose-effect curves that represented linear dose-dependent decreases in the amount of milk consumed (ml) were subjected to computer assisted least-squares linear-regression analysis that provided a log dose-effect graph of the linear function with 95% confidence limits. The ED50 was defined as the dose which decreased milk consumption by 50% compared to control. The 95% confidence limits for the ED50's were determined from the graphs. The linear-regression analysis also provided slopes and standard errors from which the 95% confidence limits for the slopes were estimated. The slopes of the dose-effect curves for the combinations were tested for parallelism to the curves for the drugs alone using a t-test as described by Tallarida and Murray [35].

For analysis by the dose-addition model, isobolograms were employed and comparisons were made between equieffective doses of the individual drugs and drug combinations. The ED50 values and 95% confidence limits for screen climbing in mice and decreases from control level of milk drinking in rats were used for the construction of isobolograms. These values for the effects of the drugs alone were plotted on the appropriate linear coordinates of the isobolograph. The line connecting these "endpoints" for the drugs alone (i.e., the theoretical dose-additive line) defined the dose-combinations which would be expected to result in a 50% decrease in performance if the drugs acted in a dose-additive manner. For dose combinations, if the 95% confidence limits of the empirically determined ED50's overlapped the theoretical dose-additive line the interaction was considered dose-additive. Points significantly above the line indicated that it required higher doses of the compounds to produce the 50% effect than would have been predicted on the basis of the relative potencies. These interactions were termed infra-additive. Conversely, points significantly below the theoretical dose-additive line indicated that the measured effects occurred at lower doses than predicted. These interactions were termed supra-additive. Note that the terms infra-additive, dose-additive and supra-additive refer to interactions that are ob-

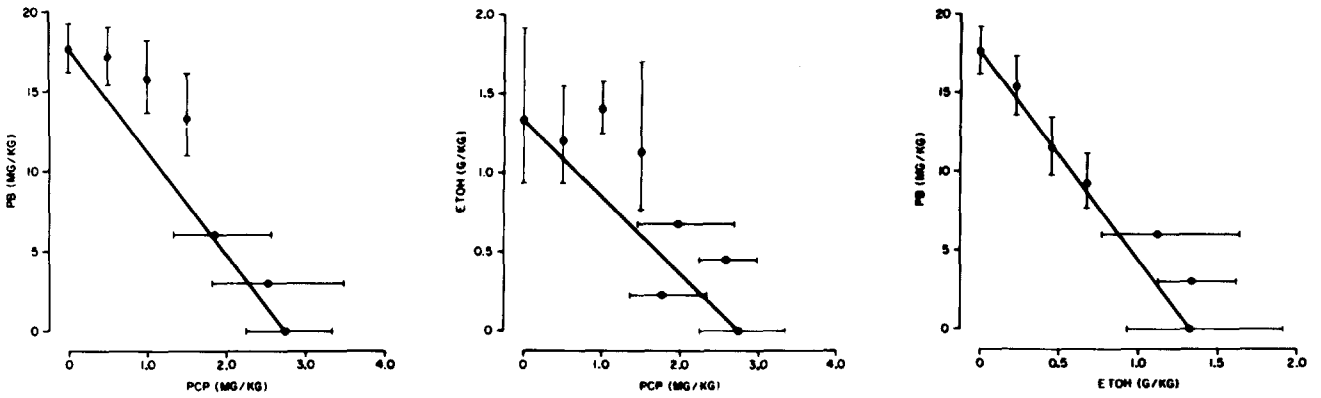

FIG. 2. Isobolographic analysis for the interactions between PCP and PB (left panel), PCP and ETOH (center panel), and PB and ETOH (right panel) tested with the inverted screen test in mice. Points with 95% confidence limits are the ED50's for the drugs alone and the combinations. The diagonal line connecting the ED50's of the drugs alone represents combinations that are predicted by the dose-addition model to produce the same effects.

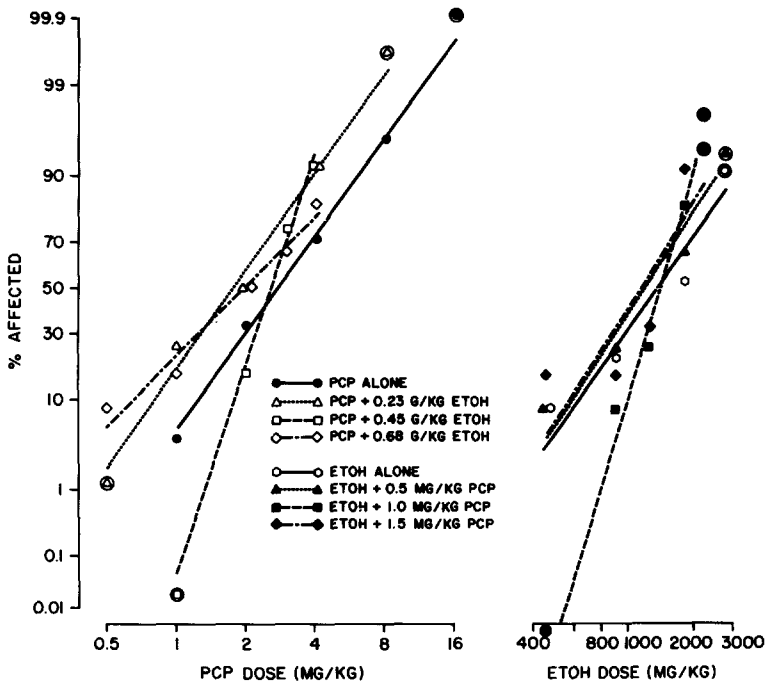

FIG. 3. Dose-effect functions for PCP, ETOH, and combinations tested with the inverted screen test in mice. Points are the observed effects of various doses or combinations; circled points are the estimated effects for doses which resulted in 0 or 100% of the mice being unable to climb to the top of the screen; lines were fitted by a computer approximation [3] of the method of Bliss [7].

served when homergic drug combinations result in leftward shifts in the dose-effect curves. If a rightward shift occurs the interaction would be termed antagonism. If the ED50 for a drug combination that resulted in antagonism were plotted on an isobolograph the point would be outside the bounds of the coordinates set up by the "endpoints" of the theoretical dose-additive line (see [39] for review).

RESULTS

Mouse Studies

PCP and PB. Dose-effect curves for PCP alone, PB alone, and combinations of PCP and PB determined using the mouse screen test are presented in Fig. 1. Doses of 3.0 and 6.0 mg/kg PB, which would be expected to have less than a

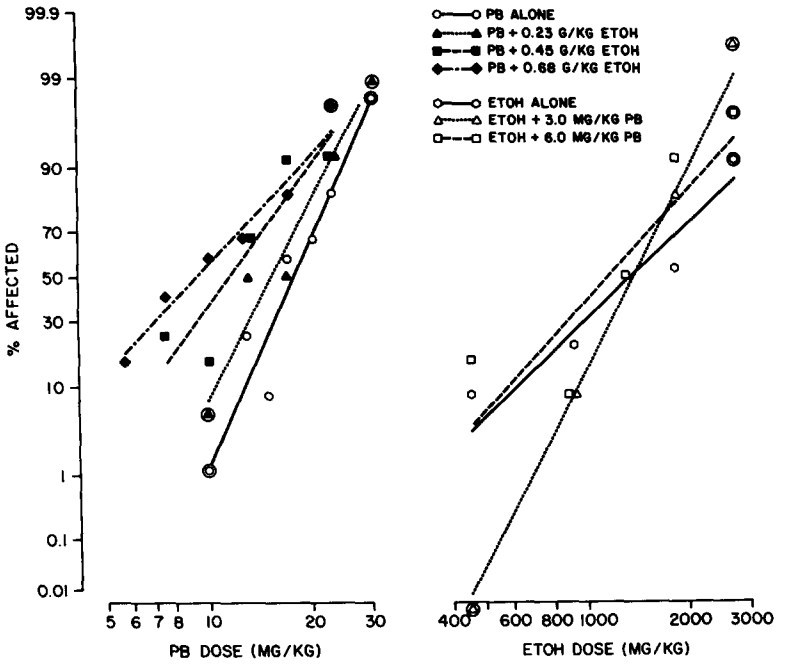

FIG. 4. Dose-effect functions for PB, ETOH, and combinations tested with the inverted screen test in mice. Points are the observed effect of various doses or combinations; circled points are the estimated effects for doses which resulted in 0 or 100% of the mice being unable to climb to the top of the screen; the lines were fitted by a computer approximation [3] of the method of Bliss [7].

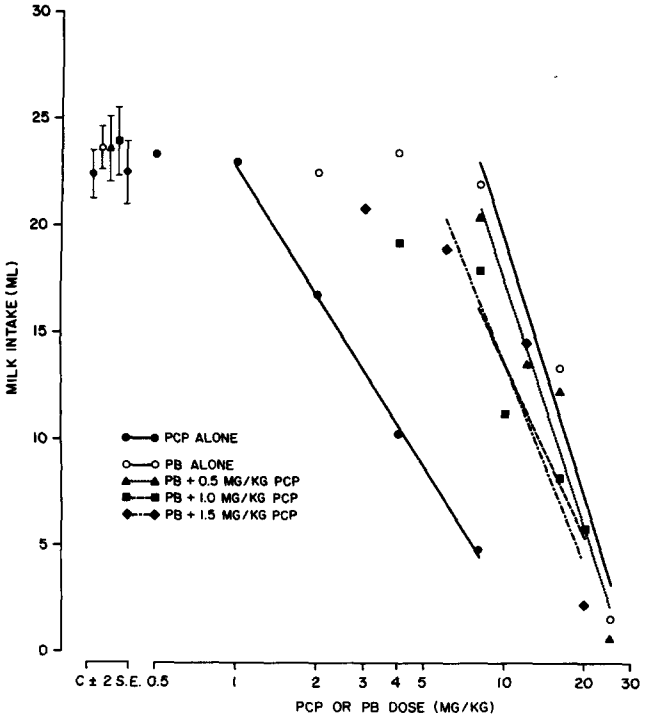

FIG. 5. Dose-effect functions for PCP, PB, and combinations of fixed-doses of PCP with PB tested with the milk consumption test in rats. Control (C) data (±2 S.E.) are group means for vehicle (saline) tests obtained during the determination of each dose-effect curve. Points are the average milk intake (ML) for the group (N=12). Functions were determined by linear-regression analysis of the linear portions of the dose-effect curves.

0.01% effect given alone (as determined by extrapolation of the PB alone dose-effect curve), when combined with PCP, caused the PCP dose-effect curve to be shifted progressively to the left and the slope to be decreased. However, these effects were not statistically significant. Similarly, the dose-effect curve for PB was progressively shifted to the left by fixed-doses of 0.5, 1.0 and 1.5 mg/kg PCP, which would be expected to have 0.3, 5.0 and 17% effect, respectively, when given alone (as determined from the dose-effect curve for PCP alone). The only statistically significant shift for the PB curves was for PB combined with 1.5 mg/kg PCP, and the three curves were parallel to the dose-effect curve for PB alone.

Dose-addition analysis for the interaction between PCP and PB is depicted using an isobologram (Fig. 2, left panel). The combination of 0.5, 1.0 and 1.5 mg/kg PCP with doses of PB resulted in infra-additive interactions and the interactions for PCP combined with 3.0 and 6.0 mg/kg PB were additive.

PCP and ETOH. Figure 3 shows the dose-effect curves for PCP alone, ETOH alone, and the combination of PCP and ETOH. The lowest dose of ETOH (0.23 g/kg), would be expected to have only 0.15% effect when given alone (determined by extrapolation of the dose-effect curve for ETOH alone). When combined with doses of PCP, 0.23 g/kg ETOH caused the dose-effect curve to be shifted parallel, but significantly to the left of the dose-effect curve for PCP alone. The ED50's for the combinations of PCP with 0.45 and 0.68 g/kg ETOH (expected effects alone, 3.5 and 14%, respectively) were not significantly different from PCP alone; however, the slope for the combination of PCP with 0.45 g/kg ETOH was significantly non-parallel to the dose-effect curve for PCP alone. The ED50's for the combination of 0.5, 1.0 and 1.5 mg/kg of PCP (expected effects alone, 0.3, 5.0 and 15%,

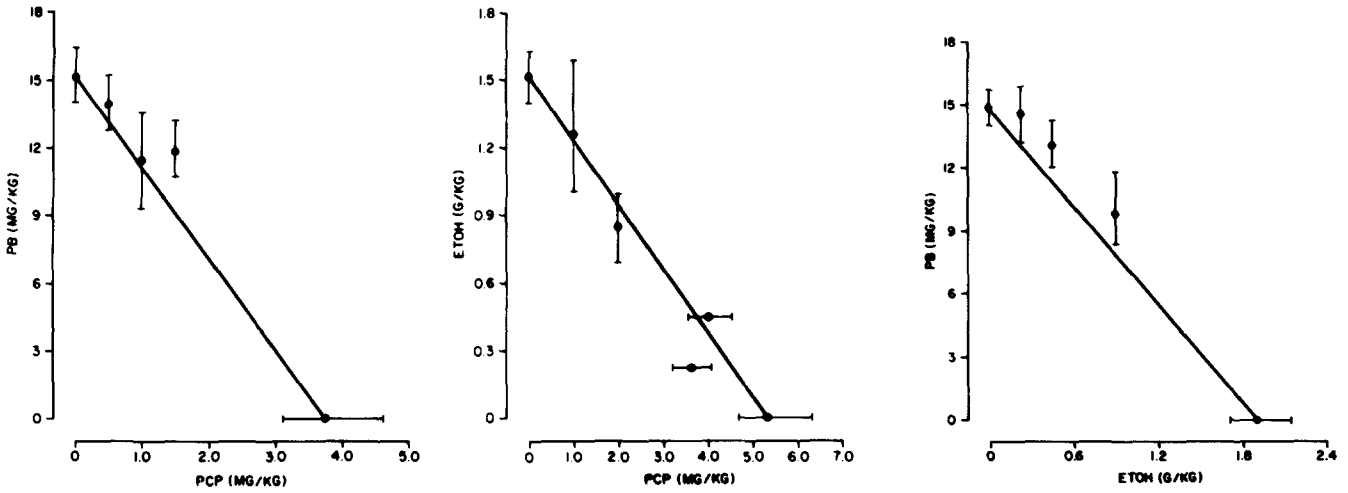

FIG. 6. Isobolographic analysis for the interactions between PCP and PB (left panel), PCP nd ETOH (center panel), and PB and ETOH (right panel) tested with the milk consumption test in rats. Points with 95% confidence limits are the ED50's for the drugs alone and the combinations. The diagonal line connecting the ED50's of the drugs alone represents the combinations that are predicted by the dose-addition model to produce the same effects.

respectively) with ETOH were not different from that of ETOH alone. However, the middle fixed-dose of PCP, 1.0 mg/kg, combined with doses of ETOH, resulted in a dose-effect curve which deviated significantly from parallel to the dose-effect curve for ETOH alone.

The isobolographic plot of the ED50 data is shown in Fig. 2 (center panel). Both the lowest dose of ETOH (0.23 g/kg) combined with doses of PCP and the lowest dose of PCP (0.5 mg/kg) combined with doses of ETOH resulted in dose-additive interactions, the confidence limits crossing over the theoretical dose-additive line. At higher doses of PCP (1.0 and 1.5 mg/kg) combined with ETOH, and at higher doses of ETOH (0.45 and 0.68 g/kg) combined with PCP, the isobol is bowed outward indicating infra-additive interactions.

PB and ETOH. The dose-effect curves for PB alone, ETOH alone and combinations of PB and ETOH are presented in Fig. 4. Increasing doses of ETOH, 0.23, 0.45 and 0.68 g/kg, which would be expected to have 0.15, 3.5 and 14% effect, respectively, when given alone (as determined from dose-effect curve for ETOH alone), combined with doses of PB shifted the PB dose-effect curve progressively to the left. The ED50's for the combination of PB with the two highest doses of ETOH were significantly different from the ED50 for PB alone, and the three curves for the combinations were parallel to the curve for PB alone. The combination of 3.0 mg/kg PB (expected effect alone, <0.01%) with doses of ETOH produced a dose-effect curve which was not shifted, but the slope was significantly steeper, crossing the dose-effect curve for ETOH alone at the ED50 point (1.3 g/kg). The curve for the combination of 6.0 mg/kg PB (expected effect alone, <0.01%) with doses of ETOH was somewhat steeper and shifted to the left of the dose-effect curve for ETOH alone, although these changes were not significant.

Dose-addition analysis of the ED50 data is shown in the isobologram in Fig. 2 (right panel). In general, the interactions for all the dose combinations of fixed-doses of ETOH with PB and fixed-doses of PB with ETOH were additive, except for the combination of 3.0 mg/kg PB with doses of ETOH. The 95% confidence limit for this combination does not quite cross the theoretical dose-additive line, thus this interaction was infra-additive.

Rat Studies

PCP and PB. The dose-effect curves for PCP alone, PB alone and combinations of PB with three fixed-doses of PCP are presented in Fig. 5. Fixed-doses of 0.5 and 1.0 mg/kg PCP, which would not be expected to decrease milk intake from control levels, and 1.5 mg/kg PCP, which would be expected to decrease milk intake by about 3.2 ml (as determined by using the regression line formula for the dose-effect curve for PCP alone), when combined with PB, caused the PB dose-effect curve to be shifted to the left. Only the dose-effect curve for 1.0 mg/kg PCP combined with PB was not parallel to the dose-effect curve for PB alone.

Dose-addition analysis for the PCP-PB interaction is depicted using an isobologram (Fig. 6, left panel) of the ED50 data. The interaction between 0.5 and 1.0 mg/kg PCP combined with doses of PB was additive, while the higher dose of PCP (1.5 mg/kg) combined with PB resulted in an infra-additive interaction.

PCP and ETOH. The dose-effect curves for PCP alone, ETOH alone, and the combinations of PCP and ETOH are shown in Fig. 7. Neither 0.23 g/kg nor 0.45 g/kg ETOH would be expected to decrease milk consumption from control levels if given alone (as determined by the dose-effect curve for ETOH alone). However, 0.23 g/kg ETOH when combined with doses of PCP resulted in a dose-effect curve that was shifted to the left of the PCP alone dose-effect curve to a greater degree than the dose-effect curve for 0.45 g/kg ETOH with PCP. Two fixed-doses of PCP (1.0 and 2.0 mg/kg) were tested in combination with doses of ETOH. Only the higher dose of PCP (2.0 mg/kg) would be expected to cause decreased milk consumption from control levels (0.6 ml decrease, as determined from the PCP alone dose-effect curve). Both doses of PCP in combination with ETOH resulted in dose-effect curves that were shifted, in a dose-related manner, to the left of the dose-effect curve for ETOH alone. Both curves for the combinations of PCP and ETOH were less steep than the ETOH alone curve.

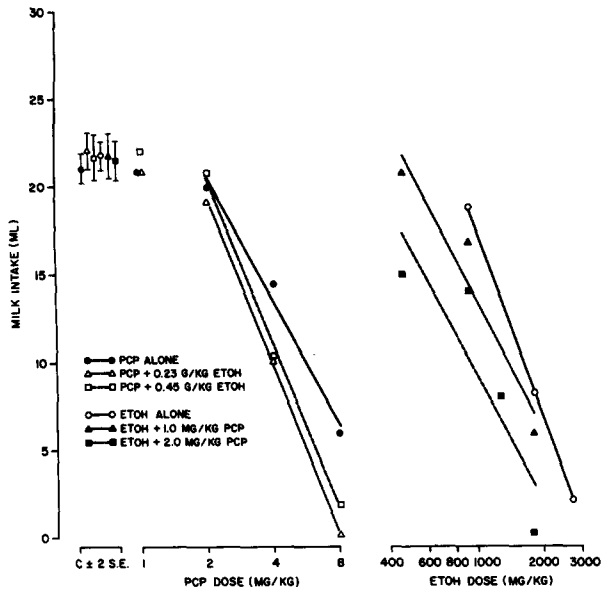

FIG. 7. Dose-effect functions for PCP, ETOH, and combinations tested with the milk consumption test in rats. Control (C) data (±2 S.E.) are group means for vehicle (saline) tests obtained during the determination of each dose-effect curve. Points are the average milk intake (ml) for the group (N=12). Functions were determined by linear-regression analysis of linear portions of the dose-effect curves.

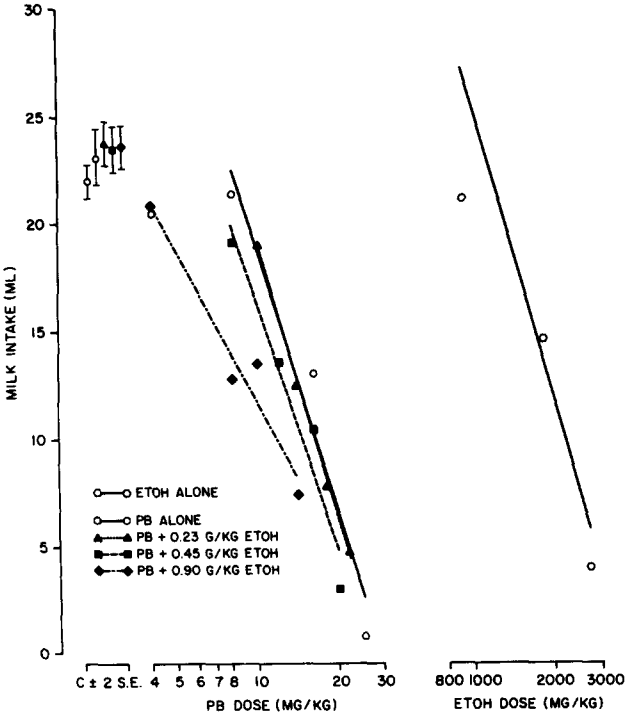

FIG. 8. Dose-effect functions for PB, ETOH, and combinations of fixed-doses of ETOH with PB tested with the milk consumption test in rats. Control (C) data (±2S.E.) are group means for vehicle (saline) tests obtained during the determination of each dose-effect curve. Points are the average milk intake (ml) for the group (N=12). Functions were determined by linear-regression analysis of the linear portions of the dose-effect curves.

Dose-addition analysis of the PCP and ETOH data is presented as an isobologram in Fig. 6 (center panel). The dose-effect curve for the combination of the low dose of ETOH (0.23 g/kg) with doses of PCP resulted in a greater shift to the left of the PCP alone curve than the higher dose of ETOH (0.45 g/kg) combination. This interaction was supra-additive by isobolographic analysis, while the higher dose combination was additive. The combinations of 1.0 and 2.0 mg/kg PCP with doses of ETOH resulted in dose-additive interactions.

PB and ETOH. The dose-effect curves for ETOH alone, PB alone, and three doses of ETOH in combination with doses of PB are shown in Fig. 8. The three doses of ETOH used in combination with PB (0.23, 0.45, and 0.90 g/kg) would not be expected to decrease milk intake relative to control (as determined from the ETOH alone curve). The dose of 0.23 g/kg of ETOH, in combination with doses of PB resulted in a dose-effect curve that was very similar to that for PB alone. The two higher doses of ETOH (0.45 and 0.90 g/kg) in combination with doses of PB resulted in dose-effect curves that were progressively shifted to the left. The slope of the curve for the combination of 0.90 g/kg ETOH with PB was less steep than the curve for PB alone.

Dose-addition analysis of the ED50 data is shown in an isobologram in Fig. 6 (right panel). The lowest dose of ETOH (0.23 g/kg) with doses of PB was slightly infra-additive, the lower 95% confidence limit for this ED50 point not quite reaching the theoretical dose-additive line. The two higher doses of ETOH (0.45 and 0.90 g/kg) combined with doses of PB also resulted in infra-additive interactions.

DISCUSSION

In both rats and mice, the homergic combinations of PCP and PB, PCP and ETOH, and PB and ETOH, usually resulted in shifts of the dose-effect curves to the left of those for the drugs alone; in no cases were shifts to the right (antagonism) observed. The degree of leftward shift was evaluated using the dose-addition model. With the mouse inverted screen test, it was generally observed that the interactions between PCP and ETOH, or PCP and PB, were most often quantitatively less (infra-additive) than the interaction between the CNS depressants, ETOH and PB (dose-additive). Interactions that are infra-additive could represent interactions of significant public health consequence [39]. Using the rat milk drinking test, the interactions between PCP and ETOH or PB were, overall, quantitatively greater (dose-additive or supra-additive) than the ETOH-PB interactions (infra-additive). That the interactions between PCP and CNS depressants can be quantitatively as large as or larger than the interactions among CNS depressants further suggests that clinically significant interactions between PCP and CNS depressants might occur in persons self-administering such combinations.

In the present study the interactions between PB and ETOH were included as a "bench mark" against which to compare the interactions of these individual CNS depressants and PCP. It is generally accepted that the clinical toxicity of barbiturates is increased by concomitant ingestion of ETOH (e.g., [21, 22, 28]). The interactions of PB and ETOH were analyzed by dose-addition analysis and the results from the mouse and rat studies differed. Using the inverted screen test with mice the interaction was dose-

additive; the isobol falling along the theoretical dose-additive line for most combinations tested (Fig. 2, right panel). This is in agreement with the isobolograms presented by Smith and Herxheimer [31] of the data of Wiberg *et al.* [41]. In contrast, the isobole for combinations of ETOH and PB for the rat milk drinking study was above the theoretical dose-additive line, thus the interaction in this case was infra-additive (Fig. 6, right panel). Gessner [19] has pointed out that the type of interaction observed depends not only on the proportions of the two compounds in the combinations, but also on the measure of drug effect employed. For example, consider the interaction of chloral hydrate and ethanol as measured by the loss of righting reflex or by lethality [20]. Chloral hydrate and ethanol were combined in a 1:1 weight ratio and isobolographic analysis revealed a supra-additive interaction when the measure of effect was loss of righting reflex. In contrast, when lethality was used to measure the interactions, the interaction was dose-additive at this dose ratio.

Relatively few studies have investigated the interactions between PCP and ETOH. The loss of righting reflex induced by ETOH was enhanced by PCP in mice [33] and rats [9]. In addition, Schüppel [30] reported that ketamine, a PCP analogue with similar actions, prolonged the ETOH-induced narcosis in rats to a similar degree as PB. Boren and Consroe [9] reported that pretreatment with ETOH caused a significant decrease in the LD50 dose of PCP in rats and vice versa. Similarly, pretreatment with PCP significantly enhanced the ETOH disruption of rotorod performance in rats [10]. There was sufficient data presented in these latter two studies to permit us to construct approximate isobolograms for dose-addition analysis. For lethality, the combination of a LD1 dose of ETOH with PCP resulted in a dose-additive interaction. Combinations of the LD20 dose of ETOH with PCP and combination of the LD1 and LD20 dose of PCP with ETOH resulted in infra-additive interactions. For rotorod performance, the single combination reported, 5 mg/kg PCP with doses of ETOH, resulted in an infra-additive interaction when interpreted by isobolographic analysis. A recent paper by Brunet *et al.* [11] reports on the interactions of PCP and ETOH in mice using several different measures. The type of interaction observed depended on the measure of effect. For example, lethal interactions were dose-additive at low ETOH doses, but became mostly infra-additive at doses of ETOH above 1.0 g/kg. When 20 and 30 mg/kg PCP were combined with ETOH, these combinations shifted the ETOH dose-effect curve to the right, thus antagonism of the lethal effects of ETOH was observed at these dose combinations. These results are in good agreement with those of Boren and Consroe [9], discussed above. In contrast to the interactions measured using lethality, the interactions for loss of righting reflex were also dose-additive at low ETOH doses, but for this endpoint shifted to supra-additive at doses of ETOH above 1.0 g/kg [11].

The rotorod test has been shown to produce results similar to the inverted screen test [4,16] employed in the present study. Isobolographic analysis of the interaction between PCP and ETOH for the mouse inverted screen test revealed an isobole which was bowed outward from the theoretical dose-additive line (Fig. 2, center panel) and was infra-additive for most of the combinations tested. These results are in contrast to the slightly supra-additive interactions observed by Brunet *et al.* [11]. While both studies are in agreement as to the direction of the interaction (in both studies the dose-effect curves were shifted leftward), they differ in regard to the magnitude of the interaction, it being less in the present study. Several factors, in addition to the fact that different test procedures were used, could have contributed to these differing results. For example, the time of testing in the present studies was 20 min post-injection, while in the Brunet *et al.* study, multiple tests were conducted, starting 6 min after injection. In addition, in contrast to the present study, a range of ETOH concentrations (holding the volume constant) was employed. ETOH has been shown to be more quickly absorbed at higher concentrations [24]. Despite these differences, the ED50's for both PCP and ETOH determined using the rotorod test (2.1 mg/kg and about 1.0 g/kg, respectively [11]) and screen test (2.7 mg/kg and 1.3 g/kg, respectively, present study) were similar. In the present study, the interaction between PCP and ETOH, while present, was not quantitatively as large as the interaction between ETOH and PB. Isobolograms with the observed ED50's falling in the area between the theoretical dose-additive line and the theoretical effect-additive isobol (this would be represented by a rectangular function connecting the ED50's for PCP and ETOH alone; ED50's for drug combinations would fall on this line if no shifts in the dose-effect curves were observed) are difficult to interpret [19]. One interpretation is that the drugs were acting by different mechanisms (independent joint action [8]) to disrupt motor performance in this task. Gessner [19] studied the interaction of two homergic drugs, ethanol and *d*-tubocurarine, which he felt would likely have unrelated mechanisms of action in causing the loss of righting reflex in mice. If independent joint action were indeed the case, he expected the ED50 values to fall along the theoretical effect-additive isobole. Experimentally, this was not observed. Instead, an infra-additive isobol similar to the one for PCP and ETOH was obtained.

In contrast to the results using the screen test with mice, are the results of interaction studies with PCP and ETOH for effects on milk drinking behavior in rats. Isobolographic analysis revealed mostly dose-additive interactions, except for the low dose of ETOH combined with PCP, which was supra-additive (Fig. 6, center panel). Of interest is the corresponding point on the inverted screen test isobolograph (Fig. 2, center panel). This point, while termed dose-additive because the 95% confidence limit crossed the theoretical dose-additive line, shows a similar trend toward supra-additivity as the upper confidence interval just crosses the dose-additive line. It would be interesting to further explore the interactions of very low doses of ETOH with PCP to see if a more robust supra-additive effect were observed. Nonetheless, for this measure in rats the interaction between PCP and ETOH was quantitatively greater than that between the CNS depressants.

A number of studies have investigated the interactions of PCP and barbiturates. In mice, PB co-administration with PCP has been shown to enhance PB lethality [14], prolong hexobarbital sleep time [33], and cause enhanced PB-induced motor incoordination [34]. In rhesus monkeys, PB's observable depressant effects were markedly enhanced by low doses of PCP [14]. In patas monkeys and pigeons PCP's disruptive effects on complex operant behavior were enhanced by PB [36,37]. The dose-effect curves for various indexes of fixed-interval performance for PB effects in rhesus monkeys were shifted to the left by co-administration of PCP [43]. In contrast, the observable depressant effects of PB and PB effects on variable-interval response rates were not shown to be enhanced by co-administration of PCP in squirrel monkeys [14,15].

The results from the mouse inverted screen test for the

interaction of PCP with PB were infra-additive for fixed-doses of PCP in combination with PB (Fig. 2, left panel), similar to the interactions seen between doses of PCP with ETOH (Fig. 2, center panel) using this same measure. The interaction between low doses of PB with PCP were dose-additive in nature (Fig. 2, left panel). In comparison to the interactions between the two CNS depressants for the mouse inverted screen test, the interactions of fixed-doses of PCP with PB were quantitatively less, while the interactions of fixed-doses of PB with PCP were at least as large as the interaction between PB and ETOH. For the rat milk drinking study the interaction of the highest dose of PCP with PB was also infra-additive, but lower doses of PCP with PB resulted in dose-additive interactions (Fig. 6, left panel). Thus the interaction of the highest dose of PCP with PB was similar to the interaction of ETOH with PB, while the interactions of the lower doses of PCP with PB were quantitatively greater for this measure.

In summary, the present experiments utilized the dose-addition model to analyse the results of drug combinations. This allowed for the characterization of shifts to the left in dose effect curves in a quantifiable manner. In the rat studies, the interactions between PCP and PB, or PCP and ETOH, were usually observed to be larger than the interactions between ETOH and PB. In the mouse studies, in contrast, the interaction between PCP and the CNS depressants were, overall, quantitatively less than the interactions between ETOH and PB. The interactions between PCP and the CNS depressants in mice were generally infra-additive. The nature of interactions depend on a number of variables including the specific dose combination, the particular effect being measured and even the route of administration (see [39] and [42] for review). Thus, it might not be surprising that the magnitude of the interactions differed in the mouse and rat studies. While the interactions differed quantitatively, they were qualitatively the same, that is, most drug combinations in both species resulted in dose-effect curve shifts to the left relative to the dose-effect curves for the drugs alone; in no cases were shifts to the right (antagonism) observed. This would suggest that the combined use of these drugs by humans may result in enhanced behavioral toxicity. At the present time it is not known if significant interactions actually occur between PCP and CNS depressants in the human abuse situation. It has been suggested that the greatly varying apparent half-life of PCP seen in drug abuse cases (11 hours to 3 days) may reflect drug interaction effects [32] and that when PCP is combined with other agents the diagnosis and treatment of overdose cases is complicated [33,34]. Given the frequency of polydrug abuse, the combination of PCP with CNS depressants such as ETOH or PB is a significant possibility. Our studies suggest the probability of important interactions which may be quantitatively as large as the interactions among CNS depressants such as ETOH and PB.

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