Pharmacology Biochemistry and Behavior, Vol. 64, No. 2, pp. 221–228, 1999

0091-3057/99/$–see front matter

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PII S0091-3057(99)00087-8

Drug Mixtures and Ethanol as Compound Internal Stimuli

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STOLERMAN, I. P., E. A. MARIATHASAN, J.-A. W. WHITE AND K. S. OLUFSEN. *Drugs and drug mixtures as compound internal stimuli.* PHARMACOL BIOCHEM BEHAV **64**(2) 221–228, 1999.—Drug discrimination methods that entail training with mixtures of drugs may shed light on polydrug abuse and on the actions of single drugs that interact with more than one receptor. In AND-discrimination procedures (drug A + drug B vs. vehicle), mixtures are discriminated primarily on the basis of their component drugs; these discriminations may be useful for testing interactions between component drugs in mixtures. The role of training dose, overshadowing and associative blocking in AND-discriminations have been investigated. For example, after prior training with midazolam, it was possible to demonstrate associative blocking of the nicotine element of the mixture stimulus, and vice versa. Using the AND-OR discriminations (drug A + drug B vs. drug A or drug B) increased pharmacological specificity considerably, and these procedures may be valuable for determining whether the effects of a novel mixture are similar to the combined effects of the training drugs. Ethanol is an example of a single drug that may produce a compound cue; rats trained to discriminate ethanol from water generalize (asymmetrically) to $GABA_A$ enhancers such as chlordiazepoxide (CDP) or pentobarbitone, to NMDA antagonists such as dizocilpine (MK-801), and to some serotonin agonists, such as trifluoromethylphenylpiperazine ($5\text{-}HT_{1B/2C}$). In addition, rats trained to discriminate mixtures of either CDP or pentobarbitone plus MK-801 generalize to ethanol. A previous history of training with MK-801 or CDP (prior to ethanol discrimination training) enhanced the MK-801-like and CDP-like effects of ethanol respectively, but associative blocking of proposed elements in the ethanol stimulus was not seen. These studies provide some support for the multielement concept of ethanol discrimination but also suggest that rules governing three-component stimuli (such as those putatively produced by ethanol) may differ from those for the two-component mixtures of drugs studied previously. © 1999 Elsevier Science Inc.

Drug discrimination Amphetamine Chlordiazepoxide Dizocilpine Ethanol Midazolam Nicotine Pentobarbitone

IT is frequently assumed that the discriminative stimuli produced by many drugs are based upon two or more elements, in which case the stimuli may be regarded as compound in nature (3). The elements may be defined either in terms of purported subjective effects that may in total form the discriminated "cue" or in terms of actions through particular neu ropharmacological mechanisms. One of the fundamental questions that arises from these concepts is whether the stimulus elements are processed independently and in parallel, or whether they merge to form a single novel, homogenous stimulus. Is the whole different from the sum of the parts? Additionally, understanding how one rather than another stimulus element comes to the fore may lead to the ability to control and manipulate the characteristics of drug-induced stimuli and thus facilitate the development of behavioral assays for particular neuropharmacological actions. Additionally, drugs are often abused in mixtures containing two or more pharmacologically diverse substances, and understanding the nature of the resultant discriminative stimuli may help to shed light on reasons for such polydrug abuse. It is not only illicit drugs such as heroin and cocaine that are frequently taken in combination; there has also been abuse of mixtures of mild, individually legal, stimulants such as caffeine, ephedrine, and phenylpropanolamine, and there is also a strong association between cigarette smoking and alcohol consumption. Synergistic pharmacological interactions between the constituent drugs may contribute to some instances of polydrug use.

An approach to the problems outlined above has been made by analysing the characteristics of the discriminative stimulus complex produced by mixtures of two drugs (binary mixtures). Such a discrimination facilitates assessment of responses to the different components of the proposed compound stimulus. For example, if tests with the elements (i.e., the separate drugs in the training mixture) alone yielded pow-

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erful discriminative effects like those of the mixture, it would imply that the components of the complex stimulus are processed independently and do not blend into a new homogenous stimulus. The results of such studies must be critically dependent upon any pharmacological interaction between the drugs in the mixture and clearly, different results might be expected with drugs that act independently, compared with those with synergistic or antagonistic effects. In addition to such pharmacological interactions, the compound stimulus will be influenced by behaviorally-determined interactions; these mechanisms have been studied in detail by associative learning theorists in their investigations on compound exteroceptive stimuli such as combinations of lights plus tones (12). Here, the relative salience of two stimuli influences the extent to which one stimulus can appear to weaken conditioning to a second stimulus presented at the same time (overshadowing), and a history of conditioning with one stimulus may weaken conditioning to a second stimulus later presented together with it (associative blocking). Insofar as mixtures of drugs (and also single drugs with multiple effects) can be considered as engendering compound interoceptive stimuli, the characteristics of such stimuli may be understood in greater depth by taking heed of what has been learned about compound exteroceptive stimuli.

This article first reviews findings from some studies on the discrimination of drug mixtures, using both substances that are thought not to interact together through known pharmacological mechanisms and that have not been subject to abuse as mixtures (e.g., mixtures of nicotine plus midazolam) and mixtures that are abused (e.g., those containing both CNS stimulant and depressant drugs, such as amphetamines plus barbiturates). The important influences to be emphasized will include the training paradigm used, the ratio between the doses of drugs in the training mixture, and the previous behavioral-pharmacological history of the subjects. Training paradigms can vary in many ways, and the particular variation that is examined below entails using a more complex (AND-OR) discrimination procedure instead of a simple AND-discrimination procedure. Examples of significant findings on the discrimination of drug mixtures will be presented, but no attempt will be made to review the area comprehensively.

In the second part of the article, some recent experiments on ethanol as a compound stimulus will be presented; these studies have attempted to apply knowledge acquired from studies on discrimination of drug mixtures to ethanol, a single drug whose discriminative effect is believed to be mediated through multiple elements. Previously, it was shown that ethanol may crossgeneralize with drugs from several distinct pharmacological classes. These drugs include both barbiturates and benzodiazepines (29,30), each of which acts as a facilitator of GABA-mediated neurotransmission, serotonin agonists acting at the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_3 subtypes of serotonin receptor (7,9,32), and drugs acting as noncompetitive antagonists at *N*-methyl-D-aspartate (NMDA) receptors (8, 28,30). These findings, considered together with studies on the discrimination of drug mixtures (15,17,36,37), led to the hypothesis that ethanol typically engenders a compound stimulus comprised of several neuropharmacologically distinguishable elements. However, the cross-generalizations between ethanol and drugs thought to mimic different elements of the ethanol stimulus are often incomplete (partial or asymmetrical generalizations). Whereas ethanol cross-generalizes fully to $GABA_A$ enhancers, certain serotonergic agonists and NMDA antagonists, animals trained to discriminate drugs from these classes typically generalize weakly, if at all, to ethanol (10,29). In contrast, generalizations between binary mixtures of drugs and their separate drugs have, with few exceptions, been complete and symmetrical(15–17, 34–37, 6).

Experiments will be described that attempted to deal with the issue of asymmetrical generalizations in ethanol discrimination by examining generalization from mixtures of different drugs to ethanol. Experiments are also presented in which an attempt was made to manipulate the characteristics of an ethanol stimulus using an approach developed in studies of drug mixtures. This approach capitalized upon the ability of a previous history of training to discriminate one drug to weaken discriminative responses to a second drug trained together with it in a mixture at a later stage (34); this effect was interpreted as associative blocking, and if it can be demonstrated with ethanol, it would support the concept that the ethanol stimulus obeys the "rules" developed for compound exteroceptive stimuli and for binary drug mixtures; such findings would strengthen the evidence that the ethanol stimulus is itself compound in nature.

METHOD

Male hooded rats were housed individually in rooms maintained at 20–22°C with a regular lighting cycle. Throughout the experiments they were fed restricted amounts of food to maintain their weights at about 80% of normal. Standard experimental chambers (Campden Instruments) were contained in sound-insulated, ventilated enclosures. The chambers were fitted with two retractable levers separated by a recess into which 45-mg pellets of food could be presented. The experiments were controlled by software (Paul Fray Ltd, Cambridge, UK) running on computers in an adjacent room.

Rats were trained according to procedures described previously (16,17). After 3 weeks of preliminary training to establish a baseline of responding under an FR-10 schedule, both levers were made available simultaneously and discrimination training began. The AND-discrimination procedure entailed training rats to discriminate a mixture of two drugs from saline. The AND-OR procedure involved training to discriminate a mixture of two drugs from either drug administered separately (16). Training took place in daily 15-min sessions, and for all experiments on drug mixtures the final schedule of food reinforcement was tandem variable interval 1-min fixed-ratio 10; under this schedule, food was presented following the 10th consecutive response on the correct bar after a randomly determined interval averaging 1 min (37). For experiments on ethanol discrimination, the final schedule of reinforcement was tandem variable-interval 15-s FR-10 because discrimination accuracy for 1.5 g/kg of ethanol was poor when the VI was set at 1 min. Accuracy during acquisition was defined by the percentage of sessions in which the correct lever was selected in a block of 10 consecutive sessions, the correct lever being the one on which a rat made the first ten presses of a session.

Extinction tests began with rats that reached a criterion of 80% accuracy based on the lever-selection index from training sessions and drug-appropriate responding in 5-min extinction tests. The extinction test sessions were conducted twice weekly, with normal training on other days. To obtain dose–response data, tests with different drug doses took place in random order, and each treatment was tested once. In addition, control tests with vehicles were carried out. The index used to assess discriminative effects was the number of responses on the lever appropriate for the training mixture, expressed as a percentage of the total number of responses on

both levers. The total number of responses on both levers served as an index of overall response rate, and these data have been presented elsewhere (13–21,34,37).

RESULTS

Characteristics of AND Discriminations

In rats trained to discriminate a mixture of (+)-amphetamine (0.5 mg/kg SC, 15 min) plus pentobarbitone (12 mg/kg SC, 15 min) from saline, increasing doses of the mixture, with the ratio between the doses of the component drugs held constant, produced a typical, dose-related generalization gradient (17). Amphetamine administered alone increased drug-appropriate responding in a dose-related manner, and its effect at the training dose was close to that of the mixture used for training. A similar result was obtained when pentobarbitone was administered alone. Increasing the dose of either drug above the training dose further increased drug-appropriate responding, at which point there were no significant differences between the responses to the mixture or to either of its component drugs (full generalization). Very similar results were obtained in later experiments with mixtures of amphetamine plus pentobarbitone (16,21), as illustrated in Fig. 1. In the initial experiments, the response to each dose of the mixture could be predicted by combining the probabilities of responses to its component drugs tested alone (17), suggesting that the interaction between the two drugs was additive in nature. In the later studies, tests with slightly different dose combinations of amphetamine plus pentobarbitone were suggestive of small degrees of synergism (16,21). The magnitude of these effects also depended upon the doses of amphetamine and pentobarbitone in the training mixture (21).

Under AND-discrimination conditions it was found that the ratio between the doses of the two drugs in the training mixture was a major influence on the outcome. This dose ratio-dependent effect was attributed to the action of the psychological process of overshadowing, rather than to a pharmacological interaction. This proposition was supported by experiments in which the dose of one drug in a training mixture was varied, while the dose of the second drug was held constant. Rats were trained to discriminate mixtures containing 0.4 mg/kg of (+)-amphetamine plus varying amounts of pentobarbitone and then generalization tests with the separate drugs were carried out. As the training dose of pentobarbitone was increased progressively from 5 mg/kg to 20 mg/kg, the response to the (varying) training doses of pentobarbitone increased. In addition, the response to the (constant) training dose of amphetamine decreased from about 92.5% drug-appropriate responding to less than 22.2% (Fig. 2); thus, pentobarbitone overshadowed amphetamine (17). The preceding studies were carried out using within-group designs where sequence effects may have had a confounding influence. However, very similar results have been obtained in between-group experiments (15), where increasing doses of midazolam progressively overshadowed the response to a fixed dose of nicotine.

Separate experiments indicated the absence of any marked pharmacological interactions between the drugs in the training mixtures (15,17); additionally, the characteristics of discriminations based on an agonist–antagonist mixture (nicotine plus mecamylamine) were also examined and were found to differ in several ways from those of the mixtures

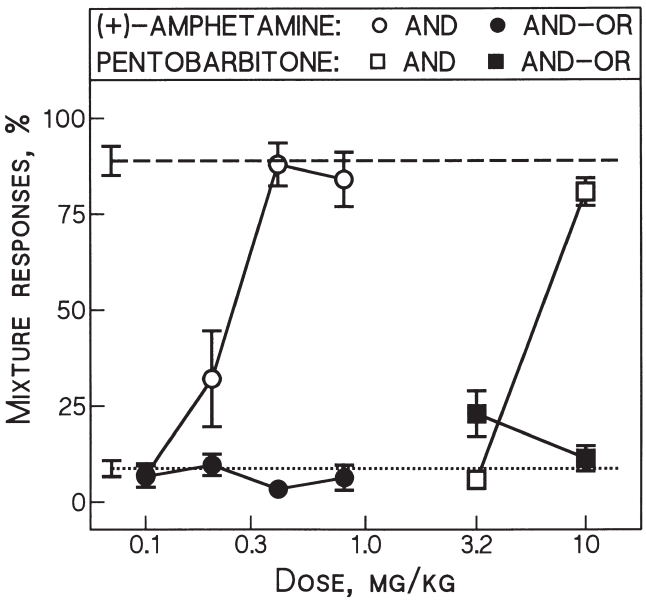

FIG. 1. Dose–response relationships for the discriminative stimulus effects of amphetamine and pentobarbitone in rats trained with AND- and AND-OR discrimination procedures (n = 8). Training doses were 0.4 mg/kg (SC, 15 min) of amphetamine and 10 mg/kg (SC, 15 min) of pentobarbitone. Horizontal dotted and broken lines present pooled responses after saline and the training mixture, respectively. Abscissa, drug dose in mg/kg; ordinate, mixture-appropriate responses in 5-min extinction tests as percentages of total responses (means ± SEM). Overlapping SEM and those smaller than diameter of symbols are omitted; based on data of Mariathasan and Stolerman, 1994 (16).

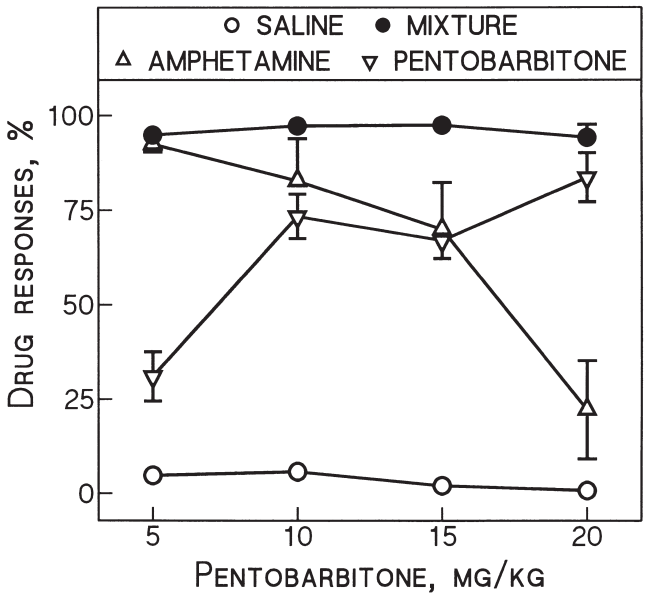

FIG. 2. Discriminative stimulus effects of amphetamine and pentobarbitone in rats trained to discriminate mixtures of these drugs from saline; based on results of Mariathasan and Stolerman, 1991 (17). Responses to the drugs are shown for successive sets of extinction tests carried out while stimulus control was maintained by mixtures containing the doses shown of pentobarbitone (n = 12). Dose of (+)-amphetamine was 0.4 mg/kg throughout. Other details as for Fig. 1.

used in the studies on overshadowing (14). First, with the agonist–antagonist mixture, the antagonist impaired acquisition of stimulus control; in contrast, midazolam did not impair acquisition. Second, using small doses of mecamylamine in training produced a shift to the left in the subsequent dose–response curve for nicotine alone and the larger doses of mecamylamine flattened the curve (with loss of stimulus control by both drug and nondrug states); with nicotine plus midazolam, there was only a downward shift in the dose–response curve for nicotine, and there was no loss of stimulus control by the nondrug state. Third, training with mecamylamine plus nicotine retarded development of tolerance to the response rate reducing effect of nicotine, whereas midazolam had no effect on the development of tolerance. Finally, there was no generalization from nicotine plus mecamylamine to mecamylamine alone, but there was dose-related generalization from nicotine plus midazolam to midazolam alone (14). These distinctions support the view that the distinct pharmacological and behavioral mechanisms can engender interactions between drugs in mixtures used for training. The nature of the interaction appears to be determined primarily by the types of drug used for training, although more extensive investigations may reveal situations where the two classes of mechanism can coexist.

Characteristics of AND-OR Discriminations

In these procedures, subjects are trained to discriminate mixtures of drugs from either component drug administered alone (16,33). In most experiments of this type, vehicle administration has produced responding appropriate to the single-drug states, suggesting that the subjects learned to discriminate the presence of the drug mixture from its absence. This resulted in a fundamental difference (in comparison with the AND-discrimination procedure) in the characteristics of drugs needed to elicit mixture-appropriate responding; under AND-OR conditions, no dose of either training drug administered separately increased mixture-appropriate responding above control (saline) levels. To illustrate this point, Fig. 1 provides a direct comparison of AND- and AND-OR discriminations involving mixtures of (+)-amphetamine (0.4 mg/kg SC, 15 min) plus pentobarbitone (10 mg/kg SC, 15 min). Based on these data, it was proposed that the AND-OR procedure engendered a more specific discrimination and several experiments designed to test this proposal were carried out.

In the first study of this type, two groups of rats were trained to discriminate mixtures of (+)-amphetamine (0.4 mg/kg) plus pentobarbitone (10 mg/kg) under the AND- and the AND-OR discrimination procedures. Three series of generalization tests were then carried out, entailing the administration of (a) the novel single drugs nicotine, midazolam, cocaine, caffeine, and ethanol; (b) mixtures in which one novel drug was administered together with the training dose of one of the training drugs; and (c) mixtures in which two novel drugs were administered. Throughout these studies, in every instance where comparisons were made, generalization was greater or occurred at lower doses under AND- than under the AND-OR discrimination procedure. The study yielded extensive evidence supporting the hypothesis that the AND-OR discrimination procedure increases the specificity of discriminations based on drug mixtures. Fuller accounts of these experiments have been given elsewhere (20).

A similar series of studies (Stolerman, Mariathasan, and White, unpublished data) has been carried out in subjects trained to discriminate mixtures of nicotine (0.4 mg/kg SC, 15 min) plus midazolam (0.15 mg/kg SC, 15 min). Three series of generalization tests were then carried out, entailing the administration of (a) the single drugs amphetamine and pentobarbitone; (b) mixtures in which amphetamine and pentobarbitone were substituted for the training doses of nicotine and midazolam, respectively, with the second drug held constant as in training; and (c) mixtures in which two novel drugs were administered (i.e., amphetamine and pentobarbitone). Under AND-discrimination conditions, there was partial generalization to 0.4 mg/kg of amphetamine (49.9 ± 8.4%) and to 10 mg/kg of pentobarbitone (63.7 ± 7.8%) when each drug was administered singly. With the AND-OR discrimination, there was weaker generalization to either amphetamine (33.6 ± 11.7%) or pentobarbitone (37.0 ± 11.3%). In "single substitution" tests in the AND-discrimination procedure, there was full generalization to mixtures of amphetamine plus midazolam (97.1 ± 1.5%) or of nicotine plus pentobarbitone (89.1 ± 3.7%); under AND-OR conditions, there was no significant generalization to the same mixtures (maximum effect 34.0 ± 14.0%). In "dual substitution" tests, a mixture of amphetamine plus pentobarbitone produced full generalization under AND-discrimination conditions (89.5 ± 4.4%), and partial generalization (50.5 ± 9.1%) in the AND-OR procedure. These results also supported the hypothesis that discriminations maintained under AND-OR procedures were more specific than the simpler AND-discriminations.

Studies with antagonists were also carried out to test the idea that the AND-OR training method enhances the pharmacological specificity of discriminations (19). Rats were trained to discriminate a mixture of nicotine (0.4 mg/kg SC) plus midazolam (0.2 mg/kg SC) from saline (AND discrimination) or to discriminate the mixture from either drug alone (AND-OR discrimination). After discriminations were acquired to 80% accuracy, the nicotine antagonist mecamylamine (0.03–1.0 mg/kg SC) and the benzodiazepine antagonist flumazenil (0.32–10 mg/kg IP) were tested on the response to the mixture of nicotine plus midazolam. The antagonist effects of either mecamylamine or flumazenil given alone were more marked in rats trained under the AND-OR procedure than in rats trained on the AND discrimination. Similarly, the antagonist effects of mixtures of mecamylamine plus flumazenil were much more potent under the AND-OR than under the AND discrimination procedure. In the AND discrimination procedure, 0.32 mg/kg of mecamylamine plus 3.2 mg/kg of flumazenil produced full block; in the AND-OR procedure, the corresponding doses were no greater than 0.032 and 0.32 mg/kg of mecamylamine and flumazenil, respectively. The AND-OR method, therefore, reduced the dose of the antagonist mixture needed to produce complete block by a factor of about 10, compared with the AND discrimination. Such reductions in doses of antagonist are considered to reflect an increased specificity of drug discriminations (2) and, therefore, they support the hypothesis of increased specificity of AND-OR discriminations (16).

Previous History in AND Discrimination

At quite an early stage in studies of the AND-OR discrimination procedure, it became apparent that the striking effects of training in this way persisted for a prolonged period even when the training procedure was changed to an AND discrimination using the same doses of the drugs (16,33). Such findings provided initial evidence for a pronounced effect of previous history on the characteristics of drug mixture discriminations. In later studies, the effect of previous history was analyzed in a different way in the AND discrimination procedure (34).

These studies employed two phases of training, with several groups of subjects trained simultaneously (n = 10). In phase I, different groups of subjects were trained to discriminate either nicotine (0.4 mg/kg SC, 15 min) or midazolam (0.15 mg/kg SC, 5 min) from saline; these groups of animals acquired their respective discriminations and showed 88–93% drug-lever selection when the mixture was administered after 30 training sessions. A control group received sham training with saline injections. In sham training, responding on one of the two levers was reinforced but there was no discriminative cue to indicate which lever was correct. As expected, no acquisition took place in the sham-trained group, and accuracy remained close to the chance level (50%). Phase II training then commenced; for the remainder of the study, all groups were trained in an identical manner to discriminate a mixture of nicotine (0.4 mg/kg) plus midazolam (0.15 mg/kg) from vehicle and after 30 sessions of such training, all groups had acquired the new discrimination to an accuracy of approximately 95%. Dose–response curves for nicotine and midazolam were then constructed in all groups, and are shown in Fig. 3. The results from the sham-trained control group showed partial generalization to both nicotine and midazolam, and each of these drugs contributed in approximately equal measure to the compound cue produced by the drug mixture. The findings in animals trained previously to discriminate each stimulus element of the mixture were very different. After a previous history (phase I) of training to discriminate nicotine, midazolam did not contribute significantly to the mixture stimulus acquired in phase II, which was, in this case, based entirely on an enhanced nicotine-like stimulus element. Contrastingly, after a previous history (phase I) of training to discriminate midazolam, nicotine did not contribute significantly and the mixture stimulus (phase II) was based entirely on an enhanced midazolam-like element (34). Thus, appropriate behavioral-pharmacological histories of exposure to training drugs can almost totally determine which elements are dominant in the compound stimulus produced by a mixture of drugs.

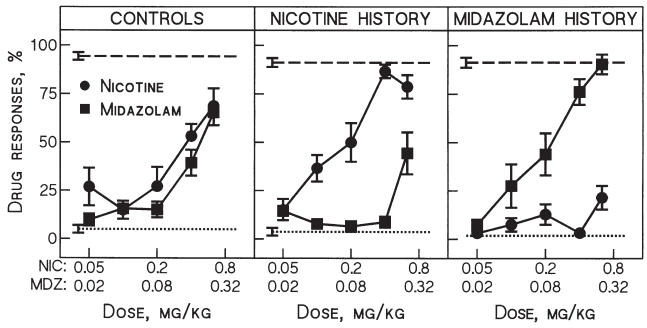

FIG. 3. Discriminative stimulus effects of nicotine and midazolam in three groups of mixture-trained rats after previous histories of sham or single-drug training (n = 10). In phase I, rats were trained in the two-lever procedure without any drug cues (sham-trained control group) or with nicotine (0.4 mg/kg SC, 15 min) or midazolam (0.15 mg/kg SC, 5 min) . In phase II, all groups were trained to discriminate a mixture of the same doses of nicotine plus midazolam from saline (AND-discrimination) and then the dose–response data shown were collected. Other details as for Fig. 1; based on results of Stolerman and White, 1996 (34).

Initial Analyses of Ethanol as a Compound Stimulus

The findings with drug mixtures provided the basis for an approach for studying the discriminative stimulus properties of the single drugs that are thought to act through multiple receptor mechanisms. Specifically, it was proposed that rats trained to discriminate mixtures of drugs that mimic the stimulus elements in the ethanol stimulus should generalize to ethanol itself. Secondly, it was proposed that the stimuli engendered by such drugs may be susceptible to manipulation by first training subjects to discriminate a different drug that is thought to mimic one element in the stimulus complex produced by the target drug (phase I); then, in phase II, conventional discrimination training for the target drug is instituted. After this drug has acquired stimulus control over behavior, it is expected that the contribution from the stimulus element that resembles the phase I training drug should be dominant and that other stimulus elements should be attenuated (through associative blocking). These ideas have been examined in studies where ethanol serves as the target drug.

The first of the present experiments with ethanol established the training method for ethanol and tested for evidence of multiple stimulus elements by means of generalization tests with some drugs used in previous studies of ethanol discrimination. Training procedures were modified from those of Grant and colleagues (7–9). Ethanol (1.5 g/kg PO, 25 min) was administered orally with a steel catheter. After stimulus control by ethanol was established, dose–response curves were constructed for (+)-amphetamine, CDP, MK-801, pentobarbitone, and the 5-HT_1 agonist RU-24969 (5-methoxy-3(1,22,3,4-tetrahydro-4-pyridinyl)-1H-indol succinate). Results showed full generalization to CDP (5 mg/kg IP, 15 min), pentobarbitone (10 mg/kg SC, 25 min) and MK-801 (0.08 mg/kg IP, 25 min). There was partial generalization (40–50%) to RU-24969 (0.1–1.0 mg/kg IP, 30 min), and no generalization to amphetamine (0.03–0.6 mg/kg SC, 15 min); response rate suppression prevented the collection of data for the discriminative effects of larger doses of RU-24969 and amphetamine. The generalizations to CDP, pentobarbitone, and MK-801 confirm previous findings, and are interpreted as evidence for both $GABA_A$-like and NMDA antagonist-like elements in the ethanol stimulus. The lack of response to amphetamine suggests that the ethanol cue was not entirely nonspecific in nature. It has been reported previously that the ethanol (1.5 g/kg) stimulus can generalize fully to RU-24969 (9), and the present lack of generalization was unexpected; however, in previous work it was possible to test much larger doses of RU-24969 before responding was suppressed, and it may be this dosage difference that accounts for the discrepancy in the findings for discriminative effects.

A subsequent study employed rats (n = 10) trained to discriminate a mixture of CDP (5 mg/kg IP) plus MK-801 (0.08 mg/kg IP) from vehicle (AND discrimination). This discrimination was acquired readily, and generalization testing with ethanol yielded a maximum response of 76.4 ± 3.6% compared with 84.6 ± 9.0% after the training mixture. This marked generalization occurred at an ethanol dose of 3 g/kg (PO) that also reduced response rates drastically and allowed discriminative effects to be assessed in eight rats only. Individually, at the training doses CDP and MK-801 produced 40.0 ± 9.3 and 44.1 ± 11.9% mixture-appropriate responding. In rats (n = 11) trained to discriminate a mixture of pentobarbitone (8 mg/kg SC) plus MK-801 (0.08 mg/kg IP) from vehicle, ethanol in a dose of 3.0 g/kg produced no more than 33.4 ± 3.5% mixture-appropriate responding. The training doses were then changed to pentobarbitone (12 mg/kg) plus MK-801

(0.04 mg/kg) to reduce the NMDA-antagonist element in the mixture stimulus. Ethanol (3 g/kg) then produced 74.5 ± 5.3% mixture-appropriate responding compared with 95.4 ± 2.7% after the training mixture; again, this dose of ethanol markedly reduced response rates but discrimination data were obtained in all 11 rats. A preliminary account of these studies was published previously (26).

Influence of Previous History on Ethanol Discrimination

These studies employed two phases of training, with four groups of subjects trained simultaneously (n = 9–10), but results for only three of these groups are summarized here. In phase I, different groups of subjects were trained to discriminate either CDP (5.0 mg/kg) or MK-801 (0.08 mg/kg) from saline; these groups of animals acquired their respective discrimination to approximately 80% accuracy after 30 training sessions. A control group received sham training with saline injections. As expected, no acquisition took place in the sham-trained group, and accuracy of lever-selection remained close to the chance level (50%). Phase II training then commenced; for the remainder of the study, all groups were trained to discriminate ethanol (1.5 g/kg PO) from vehicle, and after 30 sessions of such training, all groups had acquired the new discrimination to an accuracy of approximately 95%. Dose–response curves for ethanol, CDP, and MK-801 were then constructed in all groups.

The ethanol dose–response curves for ethanol were similar in all three groups (ED_{50} values of 0.479, 0.517, and 0.440 g/kg for rats with previous CDP, MK-801, and sham training). In the sham-trained control group, CDP produced dose-related partial generalization reaching a maximum of 75% ethanol-appropriate responding at 5 mg/kg. Similarly, MK-801 in a dose of produced 75% ethanol-appropriate responding at 0.08 mg/kg. The findings in animals trained previously to discriminate each stimulus element of the mixture were rather different. The 5 mg/kg training dose of CDP yielded 94.5 ± 1.5% and 55% ethanol-appropriate responding after previous histories (phase I) of training to discriminate CDP or MK-801. The dose–response (phase II) tests with MK-801 yielded maximum generalizations of 95%, regardless of whether rats had a previous history of training with CDP or MK-801. However, the generalizations to smaller (0.025–0.05 mg/kg) doses of MK-801 were greater in rats previously trained on MK-801 than in rats previously trained on CDP.

The preceding results clearly indicate that after a history of training to discriminate CDP, ethanol discrimination training resulted in a stimulus with a relatively strong CDP-like element. Conversely, after a history of training to discriminate MK-801, ethanol discrimination training resulted in a discrimination with a relatively strong MK-801-like element. However, in both cases there were strong residual responses to the alternative drugs that were not appreciably below the corresponding effects in the sham-trained control group.

DISCUSSION

The conclusion from the majority of studies carried out to date on the discrimination of drug mixtures from the nondrugged state (i.e., the AND-discrimination paradigm) is that mixtures of two dissimilar drugs are discriminated largely on the basis of independent, parallel processing of their component drugs; mixtures are rarely, if ever, discriminated as unique and novel homogenous stimuli. The contribution of the stimulus element derived from each training drug depends on both pharmacological factors (e.g., the ratio of the doses of the two substances used for training) and on behavioral variables (e.g., the impact of overshadowing of one stimulus by another). Results supporting such interpretation have been reported for mixtures of amphetamine plus pentobarbitone (16,17); nicotine plus midazolam (37), nicotine plus morphine (35), nicotine plus ethanol (5,18), pentazocine plus tripelennamine ([Ts and blues; (35)], phentermine plus fenfluramine (31), cocaine plus heroin [*speedballs*; (27)], diazepam plus ketamine (10), and pentobarbitone plus MK-801 (Olufsen and Stolerman, unpublished data).

Certain mixtures of drugs have been reported to support discriminations with rather different characteristics. In rats trained to discriminate mixtures of caffeine (20 mg/kg SC) plus (+)-phenylpropanolamine (PPA, 20 mg/kg SC) from saline, generalization to both caffeine and PPA was weak (25–47%) at the doses used in the training mixture, although there was almost complete generalization to larger doses of PPA (13). These results suggested that there was a weakly synergistic interaction between caffeine and PPA. These studies were instituted because of reports that mixtures of caffeine plus PPA had discriminative effects more like those of amphetamine or cocaine than did either caffeine or PPA alone (11). It has also been suggested that in pigeons, generalization from a mixture of (+)-amphetamine plus morphine to the separate drugs was incomplete (23). In monkeys trained to discriminate a mixture of heroin plus cocaine [*speedballs*; (24)], there was very little generalization to the training doses of either drug alone; however, when the dose of either drug was increased two- to threefold, there was full generalization. These results were interpreted as evidence that cocaine and heroin mutually enhanced each other's ability to produce mixture-appropriate responding. A study reported to date in abstract form only has indicated that in rats trained to discriminate a mixture of morphine (3.2 mg/kg) plus MK-801 (0.05 mg/kg) from saline, there was no generalization to any dose tested of morphine and only partial and inconsistent generalization to MK-801 (1). A ternary mixture containing caffeine, ephedrine, and phenylpropanolamine has also been examined; there was no generalization from the ternary mixture to the training doses of any of the single drugs, and it was concluded that there was a supraadditive interaction between the drugs in the mixture (6). Deliberate training with agonist–antagonist mixtures also produces marked changes in the resulting discriminations (14). Further studies with different mixtures in the AND-discrimination procedure will doubtless reveal further instances of both synergistic and antagonistic drug interactions.

Other studies have compared the specificity of different paradigms for training discriminations based on mixtures of drugs. Notably, the AND-OR discrimination procedure in which subjects learn to discriminate between the effects of a mixture from either component drug alone has been characterized more thoroughly than in previous research. In rats trained with mixtures of nicotine plus midazolam under AND-discrimination conditions, there was partial generalization to either amphetamine or pentobarbitone when each of these drugs was administered singly, whereas in the AND-OR discrimination, there was no generalization to either drug. In "single substitution" tests, a range of doses of pentobarbitone and amphetamine was coadministered with the training dose of nicotine or midazolam, respectively; there was full generalization in the AND discrimination and no generalization under AND-OR conditions. In "dual substitution" tests, mixtures of two novel substances were tested. Mixtures of amphet-

amine plus pentobarbitone produced very marked generalization under AND-discrimination conditions, but were without significant effect in the AND-OR procedure. Tests for specificity were also carried out in rats trained to discriminate mixtures of amphetamine plus pentobarbitone and studies with antagonists provided further support for this conclusion. The consistent findings of enhanced specificity under AND-OR conditions suggest that such procedures may be particularly useful for identifying neuropharmacological mechanisms underlying discriminations of drug mixtures. AND-OR procedures may be regarded as a subset of the class of drug vs. drug training methods that have often been associated with increased specificity in earlier work.

In addition to the current training procedure, the previous history of subjects can have a striking effect upon the characteristics of drug mixture discriminations. This was seen first in studies where the effects of a history of AND-OR discrimination training persisted long after the subjects were switched to the simpler AND-discrimination paradigm (16,33). The consequences of training first on a single drug and then with the same drug in a mixture with another substance also indicated the impact of different previous behavioral-pharmacological histories (34). The history effects were attributed to associative blocking (12), in which a history of conditioning to discriminate one stimulus seems to weaken conditioning to a second stimulus subsequently trained in compound with it. These history effects in blocking experiments were remarkably persistent across several months of continued discrimination training, as demonstrated more explicitly in a later study that confirmed the major findings of the original experiment, although the magnitude of effects of previous history was rather smaller in this study (25).

The investigations described above attempted to delineate some general principles that may determine the discriminative stimulus effects of many drug mixtures. Experiments were then carried out to examine possible implications of the drug mixture studies for the discrimination of ethanol, a drug thought to produce a stimulus with two or more elements. In this case, the main effect of different training histories seems to have been an enhancement of the response to the drugs used for training in phase I. The procedures, therefore, seemed to have successfully modified the characteristics of the ethanol discrimination, but not to the extent of producing the dramatic effects evident in the study of blocking in discrimination of a drug mixture. Because the different phase I procedures did not result in attenuation of the responses to the alternate drugs, there were no grounds for claiming that associative blocking of the corresponding alternate elements of the ethanol stimulus was achieved. Another interpretation of these data can be derived from studies showing that after a history of training to discriminate one drug, discriminative responses to that agent were retained despite subsequent training of responses to a second, different agent (22). These finding have implications for interpretation of the present results; the enhanced response to CDP in rats trained on ethanol after a CDP training history may have reflected retention of the original CDP discrimination rather than an increase in the CDP-like element of the ethanol discrimination. A similar argument could be made with respect to the apparent enhancement of the NMDA antagonist-like element in the ethanol stimulus. The data available at this time do not allow a distinction to be made between the two interpretations.

The studies on conventional ethanol discriminations and on drug mixture discriminations provided stronger support for the notion of ethanol as a compound stimulus. First, these studies confirmed earlier reports that ethanol can generalize to drugs of more than one pharmacological class. Second, rats trained to discriminate mixtures of a $GABA_A$ enhancer (CDP or pentobarbitone) plus the NMDA antagonist MK-801 generalized relatively strongly to ethanol; these generalizations were in striking contrast to predominantly negative results of generalization tests to ethanol in rats trained to discriminate benzodiazepines (4,10) or the NMDA antagonist ketamine (10), but it should not be overlooked that the generalizations were still partial and were only seen at doses of ethanol that drastically reduced overall response rates. Such findings confirm and extend observations (10) of similar generalization to ethanol in rats trained to discriminate a mixture of diazepam plus ketamine from vehicle. The present findings suggest that such discriminations from drug mixtures to ethanol are greatest when the training dose of the $GABA_A$ enhancer is large relative to the dose of the NMDA antagonist.

It can be concluded that the present attempt to apply lessons from the discrimination of drug mixtures to the multielement concept of ethanol discrimination has had limited success. Several factors may contribute to this outcome and deserve further investigation. The weak (asymmetrical) generalization from the phase I training drugs to ethanol may have impaired the probability of seeing associative blocking, because in drug mixture studies the single drugs used in phase I generalized fully to the mixture used in phase II (34). Another possibility is that the discriminative stimulus effects of the two phase I training drugs (i.e., CDP and MK-801) were too similar to allow for dissection of the ethanol stimulus in the way intended. However, although there are rare reports of crossgeneralization between benzodiazepines and MK-801, a negative outcome is more usual; therefore, it seems unlikely that similarities between their effects confounded the outcome. Finally, it may be the case that rules governing three-component stimuli such as those putatively produced by ethanol, (with possible $GABA_A$-enhancing, 5-HT–like and NMDA antagonist-like effects) may differ from those for the binary mixtures of drugs studied previously. Despite these difficulties, the endeavor to apply principles from mixture studies to single drugs should proceed in view of the potentially very wide range of drugs to which it may relate including, in addition to ethanol, mixed agonist–antagonist opioids such as cyclazocine, lysergic acid diethylamide, and the atypical antipsychotic clozapine.

ACKNOWLEDGEMENTS

This research was supported by NIDA Grant DA-05543 and by an MRC Studentship (K.S.O.). We thank Katherine Grant for invaluable advice relating to the studies on ethanol generally, and Michael Oglesby and colleagues for helpful discussions on drug mixture generalizations to ethanol.

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