*Pharmacology Biochemistry & Behavior*, Vol. 21, pp. 877–881, 1984.  0091-3057/84 $3.00 + .00

# Characterization of the Interaction of Pentazocine and Tripelennamine: Drug Discrimination and Mu-Receptor Binding Assay

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Received 23 February 1984

SHOOK, J. E., M. J. KALLMAN, B. R. MARTIN AND W. L. DEWEY. *Characterization of the interaction of pentazocine and tripelennamine: Drug discrimination and mu-receptor binding assay.* PHARMACOL BIOCHEM BEHAV **21**(6) 877–881, 1984.—Abuse of the combination of pentazocine (P) and tripelennamine (T) reputedly produces an opiate-like euphoria not obtainable from either drug alone. To determine if this effect is related to interactions at the behavioral or receptor levels we tested this combination in rats trained to discriminate morphine from saline and in mu-receptor binding assays. Displacement of $^3$H-DHM was compared in morphine-naive, dependent and withdrawn states to determine the importance of prior morphine exposure. The morphine training cue (3 mg/kg) generalized to P but not to T. Combinations of T (0.3 and 1.0 mg/kg) with "no effect" doses of P (1 and 3 mg/kg) resulted in greater than additive increases in morphine-like responding. $^3$H-DHM was displaced by P but not T in naive, dependent and withdrawn states. Specific dose combinations of T (1 nM) with P (1 nM, 10 nM, 100 nM) resulted in enhanced displacement of $^3$H-DHM and was not related to prior morphine exposure. We conclude that the addition of T to P increases the mu-like subjective effects of P and this effect may be due to enhanced affinity of P for the mu-receptor.

Pentazocine Tripelennamine Drug discrimination Mu-receptor Morphine-dependence

COMBINATIONS of pentazocine and tripelennamine are substitutes for heroin among some street addicts [2, 6, 8, 13]. Pentazocine, a narcotic agonist/antagonist produces both analgesia and unpleasant psychotomimetic effects [2, 6, 8]. Tripelennamine is a histamine (H1) antagonist of the ethylenediamine series with anticholinergic, antidepressant and local anesthetic activities [2, 5, 6, 13]. According to addicts, specific dose ratio combinations (i.e., 3 pentazocine: 1 tripelennamine) produce an opiate-like euphoria not obtainable from either drug alone [2,6]. The ratio of pentazocine to tripelennamine is also reported to be crucial in avoiding tripelennamine-induced seizures, as the incidence of seizures increases with increases in tripelennamine dosage [2].

There is question about the rationale and validity of the use of this combination as a substitute for heroin in opiate-dependent humans because pentazocine and tripelennamine alone and in combination do not completely substitute for morphine in opiate-dependent animals [1]. There are suggestions that tripelennamine may either enhance mu properties or depress sigma properties of pentazocine [10]. We have addressed the possibility that tripelennamine may be directly effecting the mu properties of pentazocine. We tested the combination of pentazocine and tripelennamine in the drug discrimination paradigm to determine if it was morphine-like in rats trained to discriminate morphine from saline. Drug discrimination is a means of comparing the subjective effects of different drugs [7], including narcotic analgesics [9]. In addition, there is a high correlation between compounds which are positive in this test and drugs which act through the same receptor [4]. We chose morphine as the training drug. It is a selective agonist for the mu-opiate receptor, and only those drugs which also interact with mu-opiate receptors would be expected to generalize to the discriminative stimulus properties of the morphine training cue [4]. Studies were also carried out to determine whether tripelennamine directly altered the interaction of pentazocine with mu-opiate receptors *in vitro* using $^3$H-dihydromorphine, a selective mu-opiate receptor agonist.

While most research on pentazocine and tripelennamine have concentrated on the drug-naive state, combination abuse has only been reported in known opiate-abusers. Therefore the opiate binding assays were conducted with brain tissues from morphine-naive, dependent and withdrawn mice.

### *Drugs*

Levorphanol and pentazocine were received as generous gifts from Hoffman-LaRoche and Sterling Winthrop Research Institute, respectively. Morphine and $^3$H-dihydromorphine were obtained from Malinkrodt chemicals

and New England Nuclear, respectively. Naloxone and tripelennamine were obtained from Endo Laboratories.

*Drug Discrimination*

Adult male rats (Sprague-Dawley, Dominion Laboratories, Dublin, VA) maintained at about 80% of their free-feeding weights were initially trained to lever press in a standard operant chamber on a variable-interval schedule for sweetened-milk reinforcement. Once trained to this operant task, rats were trained to press specifically the right or left lever depending on whether they were under a drug or non-drug state. Each rat was assigned a particular drug-appropriate and saline-appropriate lever, and lever assignments were counter-balanced across the entire group (n=6 to 8). Sessions were run daily and drug and vehicle injections were presented on a double-alternation schedule. On days of drug training (3 mg morphine sulfate ($MSO_4$)/kg body weight, IP, 30 min before the session) rats were reinforced for responses made only on the drug-appropriate lever. When given injections of saline only those responses made on the saline-appropriate lever were reinforced. As training progressed and throughout the entire test period, the degree of discriminative control was determined by 2.5 minute extinction tests at the beginning of every other session. No reinforcement was given during extinction testing. The percentage of total responses made on the drug-appropriate lever and the total number of responses made per minute were calculated from data recorded during the extinction test period. After discrimination stabilized at 85% or greater correct responding following consecutive saline and morphine injections, testing with novel compounds began. Only those animals meeting testing criteria of 85% or greater correct responding on the most recent morphine and saline test days were used in these experiments.

*Opiate Binding Assay*

Male ICR mice (Dominion Laboratories, Dublin, VA) weighing 20 to 30 g were divided into two experimental groups: morphine-naive and morphine-dependent. Mice in the dependent group were given unlimited access to morphine-admixed food and water as described by Tagashira *et al.* [12] for 9 days. On day 9 a group of the morphine-dependent mice was tested for tolerance on the hot plate [3] and for dependence by the naloxone-induced jumping test [14]. The remaining dependent mice were either immediately sacrificed for use in the binding assay or were switched to non-drug food for 24 hr to induce withdrawal. Withdrawn mice were assessed for withdrawal signs before they were sacrificed for use in the binding assay. Naive mice received no drug treatments prior to sacrifice.

Mouse brains (minus cerebellum) were homogenized in 5 ml of tris buffer (5 mM, pH 7.4) and diluted to a final concentration of 1 g/100 ml of buffer. For Scatchard analysis 2 ml aliquots of homogenate were incubated for 5 min at 35°C in the presence or absence of levorphanol (5 $\mu$M). Varying concentrations of $^3$H-dihydromorphine ($^3$H-DHM) were then added to the samples, followed by a 90 min incubation at 4°C. For competition studies, pentazocine, tripelennamine or combinations thereof were added to 2 ml of homogenate and were allowed to incubate for 5 min prior to the addition of $^3$H-DHM (2.5 nM) for a 90 min incubation at 4°C. Samples were then filtered on double GF/C Whatman filters and washed twice with cold tris buffer. Dried filters were shaken for 60 min with 10 ml aqueous counting scintillation fluid (Amersham Corp., Arlington Heights, IL) and counted for radioactivity. Specific binding was determined by subtracting the binding in the presence of levorphanol from the total binding. The percent of $^3$H-DHM displaced by competing ligands was determined by dividing binding displaced by pentazocine (with or without tripelennamine) by specific binding (total binding minus that in the presence of levorphanol, 5 $\mu$M). Results were analyzed statistically using Student's *t*-test.

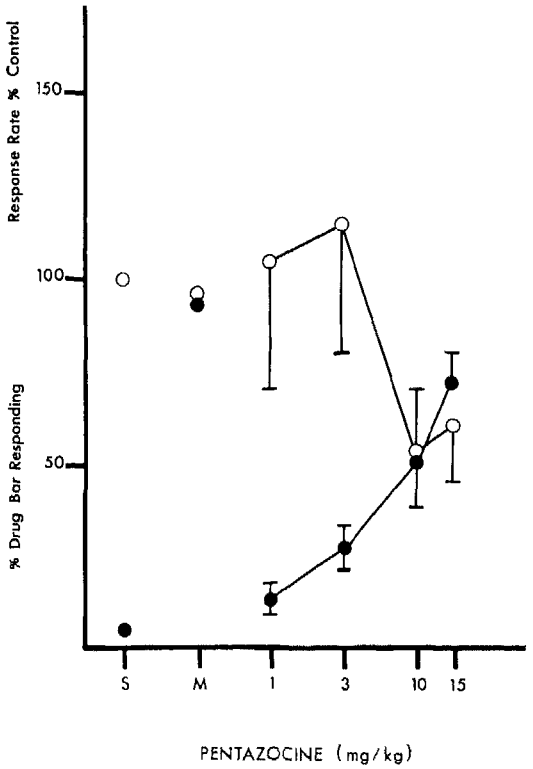


FIG. 1. Dose effect curves for pentazocine for % drug bar responding (closed circles) and response rate expressed as % of control (open circles) in rats trained to discriminate between saline (S) and 3 mg/kg morphine (M). The means±S.E.M. are presented for 8 animals. Where no S.E.M. bars are shown, the S.E.M. is encompassed by the symbol.

## RESULTS

*Drug Discrimination*

Pentazocine produced dose-related increases in morphine-like responding (Fig. 1). Doses of 1 and 3 mg/kg of pentazocine resulted in responding primarily on the saline lever and for this reason these two doses of pentazocine were chosen for testing in combination with tripelennamine. Low doses of pentazocine had no effect on response-rate (RR), whereas the higher doses suppressed RR.

Tripelennamine did not generalize to the morphine-training cue (Fig. 2) in doses up to 3 mg/kg. The lowest dose of tripelennamine appeared to suppress RR, while higher doses resulted in rates that were not different from controls. Since all three doses of tripelennamine failed to generalize to the morphine cue, they were tested in combination with pen-

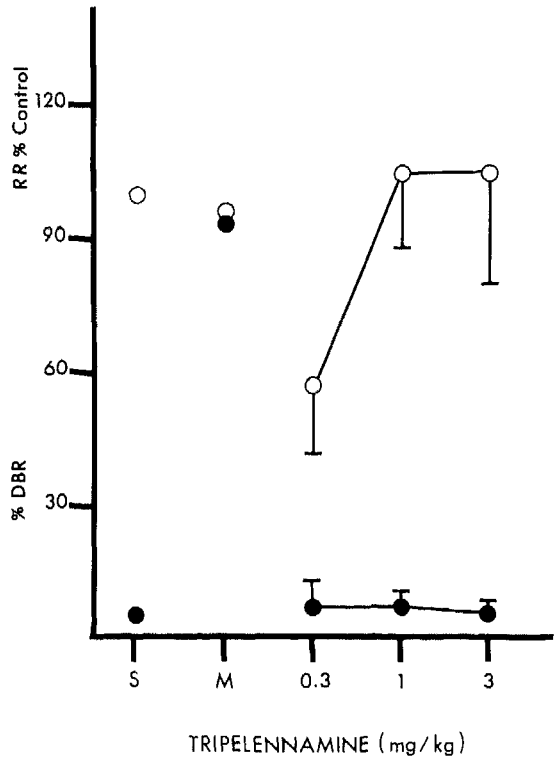


FIG. 2. Dose effect curves for tripelennamine for % drug bar responding (% DBR closed circles) and response rate expressed as % of control (RR% control open circles) in rats trained to discriminate between saline (S) and 3 mg/kg morphine (M). The means±S.E.M. are presented for 8 animals. Where no S.E.M. bars are shown, the S.E.M. is encompassed by the symbol.

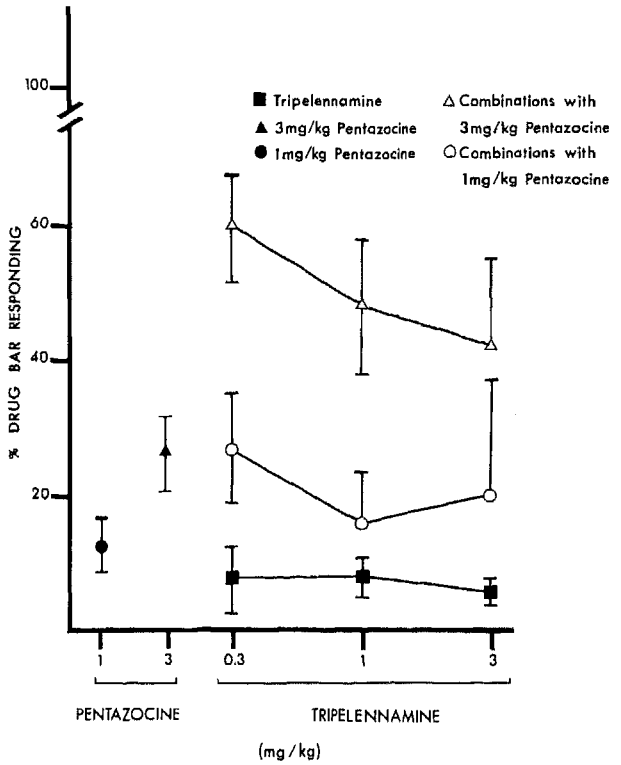


FIG. 3. Dose effect curves for combinations of pentazocine and tripelennamine for % drug bar responding. Closed figures represent the individual drugs (tripelennamine 0.3, 1.0 and 3.0 mg/kg in closed squares, pentazocine 1.0 mg/kg in closed circle, and pentazocine 3 mg/kg in closed triangle). Open figures represent combinations of all doses of tripelennamine with 1 mg/kg pentazocine (open circles) and 3 mg/kg pentazocine (open triangles). The means±S.E.M. are presented for 8 animals.

tazocine. The responding elicited by combinations of pentazocine and tripelennamine are shown in Fig. 3. Some combinations of pentazocine and tripelennamine resulted in responding similar to that produced by higher doses of pentazocine alone. Increasing doses of tripelennamine together with 3 mg/kg pentazocine produced dose-dependent decreases in percent drug-bar responding. The combinations of 3 mg/kg pentazocine plus 0.3 mg/kg tripelennamine (10:1 ratio) and 3 mg/kg pentazocine and 1 mg/kg tripelennamine (3:1 ratio) both produced greater than additive effects in percent drug-bar responding; the 10:1 ratio elicited the greatest potentiation. All other combinations presented in Fig. 3 yielded percent drug-bar responding not different from the expected additive results.

We also compared the effects of the drug combinations on RR to the effects of the individual drugs alone (data not shown). The only apparent effect on RR produced by the combination was an increase in variability. While suppression of RR occurred at the same dose combinations which also resulted in greater than additive increases in percent drug-bar responding, no other consistent trends could be established.

*Opiate Receptor Binding*

Saturation analyses of $^3$H-DHM binding (0.5 nM to 7.5 nM) were performed to determine the *in vivo* binding characteristics of $^3$H-DHM in the morphine-naive, dependent and withdrawn states. Scatchard analysis revealed linear plots with similar single binding components for each drug state. Comparison of the naive, dependent and withdrawn states demonstrates similar general dissociation constants ($K_D$) for $^3$H-DHM in all states (naive $K_D$ 3.5±0.1 nM, dependent $K_D$ 7.8±0.3 nM, withdrawn $K_D$ 3.5±0.3 nM).

In preliminary studies with naive, dependent and withdrawn states pentazocine (1 nM to 10 $\mu$M) displaced $^3$H-DHM (2.5 nM) in a concentration dependent fashion, while tripelennamine (1 nM to 1 $\mu$M) did not. The displacement of $^3$H-DHM by pentazocine was enhanced with the addition of tripelennamine, but only at speicific dose-ratios. Consequently further displacement studies were done with the three most effective combinations only.

Dose ratios (pentazocine:tripelennamine) of 1:1 (1 nM:1 nM), 10:1 (10 nM:1 nM), and 100:1 (100 nM:1 nM) were tested for displacement of $^3$H-DHM in the naive, dependent and withdrawn states (Fig. 4A, B and C). The same concentrations of pentazocine and tripelennamine alone were also tested. Tripelennamine alone did not displace $^3$H-DHM in any drug state. Pentazocine alone displaced $^3$H-DHM in all three states and the addition of tripelennamine consistently potentiated this effect. The data presented in Fig. 4A demonstrate the potentiated displacement of $^3$H-DHM by the 10:1 and 100:1 combinations in the naive state. Figs. 4B and

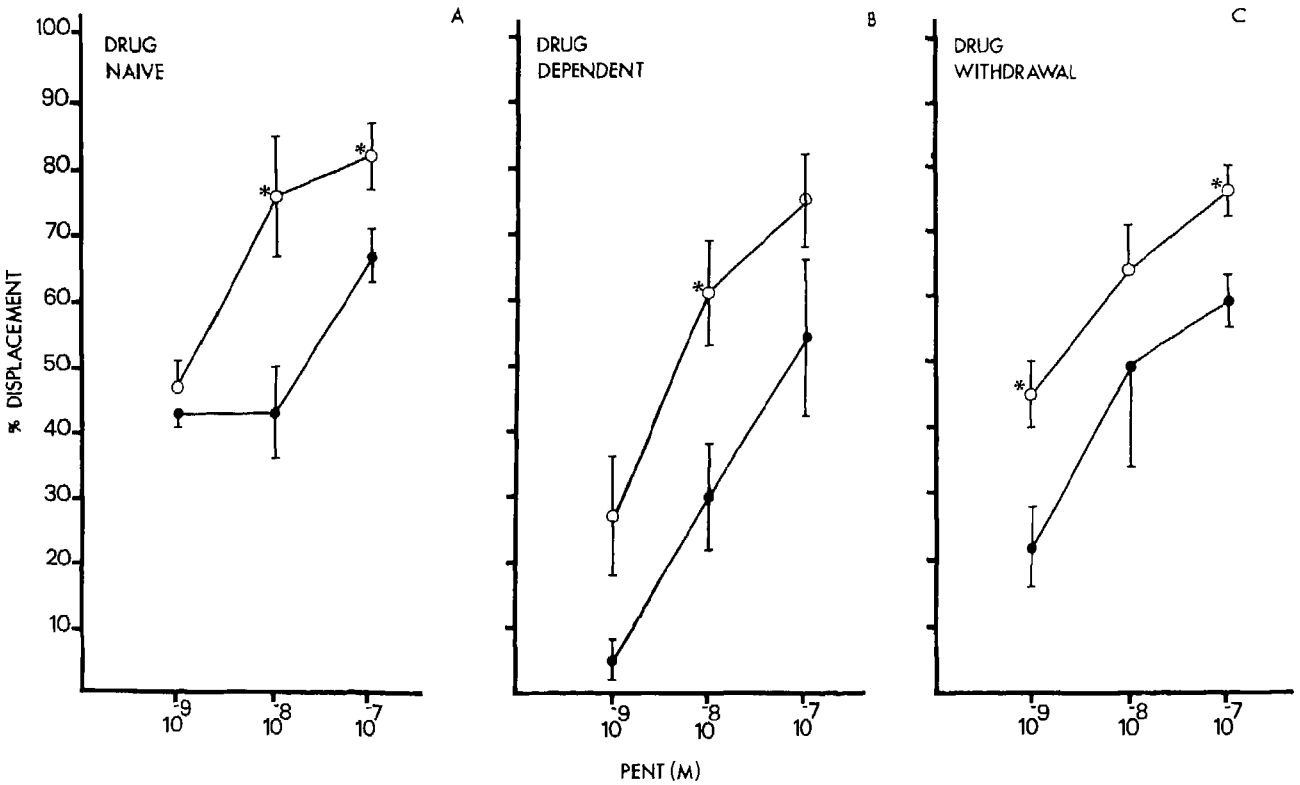


FIG. 4. % Displacement of $^3$H-DHM by pentazocine alone (closed circles) and pentazocine in combination with 1 nM tripelennamine (open circles) in the morphine-naive (4-A), morphine-dependent (4-B) and morphine-withdrawn (4-C) states. All points represent means±S.E.M. of 5 replications each performed in triplicate. Asterisks represent significant differences ($p<0.05$).

4C contain the data from the dependent and withdrawn states, and in both cases all three dose combinations resulted in greater displacement of $^3$H-DHM than did the same doses of pentazocine alone. Although not all points are significantly different, the combinations of pentazocine and tripelennamine consistently produced a greater displacement than pentazocine alone.

## DISCUSSION

Some combinations of pentazocine and tripelennamine produced greater than additive increases in morphine-like responding and displacement of $^3$H-DHM. From these data, we conclude that tripelennamine potentiates the morphine-like subjective effects of pentazocine and this may be related to enhanced mu-receptor activity. This potentiation is both dose-dependent and dose-ratio dependent. The greatest potentiation of drug-bar responding was produced by the combination of the lowest dose of tripelennamine (0.3 mg/kg) and the highest dose of pentazocine (3 mg/kg). In this paradigm, the 10:1 ratio was more potent than the commonly abused 3:1 ratio. The fact that some combinations yield greater than additive increases in drug-bar responding implies that tripelennamine makes pentazocine seem more morphine-like to the rat. This enhancement of opiate-like effects by tripelennamine also substantiates reports from human addicts that the pentazocine and tripelennamine combination is an effective substitute for heroin.

Our study is complimentary to the work of Shannon and Su [10], who showed that some combinations of pentazocine and tripelennamine acted to increase the morphine-like discriminative stimulus properties and markedly reduce the N-allylnormetazocine-like discriminative stimulus properties of pentazocine in rats trained to discriminate saline from morphine or N-allylnormetazocine in a discrete trial-shock avoidance paradigm. Also, in accordance with our findings, Shannon and Su saw greater increases in the morphine-like discriminative stimulus properties of pentazocine when it was combined with a lower dose of tripelennamine (0.3 mg/kg) than with a higher dose (3.0 mg/kg).

In contrast to our work, Shannon and Su [10] did not see enhanced displacement of $^3$H-naloxone by pentazocine in the presence of tripelennamine. This difference in findings may be related to the fact that Shannon and Su used a narcotic antagonist ($^3$H-naloxone) while we used a narcotic agonist ($^3$H-DHM), but is more likely a result of major differences between the dose ratios of pentazocine to tripelennamine that were tested. Shannon and Su studied the inhibition of $^3$H-naloxone binding in the absence and presence of 100 nM tripelennamine. This is a relatively high dose of tripelennamine. Combinations with proportionately greater amounts of tripelennamine than pentazocine are not commonly abused and are reported to produce noxious side effects, especially convulsions [2]. In our binding studies we tested 1 nM tripelennamine in combination with 1, 10 and 100 nM pentazocine, thus utilizing dose ratios which more closely

approximate the combinations abused by addicts as well as those combinations which resulted in enhancement of the morphine-like discriminative stimulus properties of pentazocine exhibited by those data presented here and by Shannon and Su [10].

Su has tested binding of pentazocine and tripelennamine to the N-allylnormetazocine binding site and has shown that both pentazocine and tripelennamine alone can displace N-allylnormetazocine binding [11]. Su hypothesized that the combination may decrease the psychotomimetic activity of pentazocine by preventing the interaction of pentazocine with the sigma receptor [11].

The findings of Shannon and Su [10], and Su [11], do not rule out the possibility that tripelennamine may affect the interaction of pentazocine with the mu receptor. And indeed, in our experiments the addition of tripelennamine to pentazocine resulted in an enhancement of $^3$H-DHM displacement in the naive, dependent and withdrawn states. Scatchard analysis of $^3$H-DHM binding in the three drug states revealed no change in the affinity of the ligand for its receptor. Likewise the displacement of $^3$H-DHM by pentazocine and the influence of tripelennamine was consistent in all drug states, indicating that combinations of pentazocine and tripelennamine may be abusable by naive persons as well as experienced users and addicts.

From these data we conclude that certain combinations of pentazocine and tripelennamine exert some molecular influence at the mu receptor *in naive*, dependent and withdrawn states to enhance pentazocine binding. This is consistent with reports from addicts that certain dose-ratio combinations result in an opiate-like state.

The data reported herein as well as that of Su [11] suggests that the synergistic opiate-like effects of the combination of pentazocine and tripelennamine at the behavioral level may be related to enhanced affinity for the mu receptor as well as antagonistic activity at the sigma receptor.

ACKNOWLEDGEMENTS

Supported by U.S.P.H.S. grants No. DA-00490, DA-01647 and T32-DA-07027.

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