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BRIEF COMMUNICATION

A Comparison of Anxiolytic and Nonanxiolytic Agents in the Shock-Probe/Burying Test for Anxiolytics

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TREIT, D. *A comparison of anxiolytic and nonanxiolytic agents in the shock-probe/burying test for anxiolytics.* PHARMACOL BIOCHEM BEHAV **36**(1) 203–205, 1990.—The effects of IP midazolam (1.0–3.0 mg/kg), pentobarbital (10–20 mg/kg), ethanol (500–2000 mg/kg), scopolamine (0.05–1.25 mg/kg), chlorpromazine (0.5–5.0 mg/kg), yohimbine (0.5–2.0 mg/kg), and pentylenetetrazol (5.0–20.0 mg/kg) were compared in the shock-probe/burying test. Consistent with results found previously for chlordiazepoxide and buspirone, the anxiolytic agents midazolam and pentobarbital decreased rats' burying behavior toward a continuously electrified (2 mA) shock-probe, and increased the number of contact-induced probe-shocks rats received. A concurrent decrease in probe-burying and increase in probe-shocks was not reliably observed after ethanol, scopolamine, chlorpromazine, yohimbine, or pentylenetetrazol. Although most of these nonanxiolytic agents produced some suppression of burying behavior at high doses, none of these drugs induced a significant increase in probe-shocks. In fact, pentylenetetrazol, which is believed to be anxiogenic, produced a significant reduction in probe-shocks. Yohimbine, another putative anxiogenic agent, was not active in the present test. In summary, concurrent increases in probe-shocks and decreases in probe-burying seem to be characteristic effects of clinically useful anxiolytic agents, which distinguish them from nonanxiolytic agents.

Anxiolytics Shock-probe/burying Midazolam Pentobarbital Ethanol Scopolamine Chlorpromazine Yohimbine

RATS shocked once from an electrified probe characteristically spray bedding material toward or over the probe [i.e., "burying" behavior (9)]. Antianxiety (i.e., anxiolytic) agents suppress this burying response in a dose-related manner, and with a relative potency that is similar to that found in clinical settings (10). The effects of anxiolytic agents on probe-burying can be distinguished from those of several nonanxiolytic agents (1, 10, 11), and do not appear to be secondary to behavioral sedation (2,10), analgesia (8), or associative deficits (2).

Similar results have been reported by Meert and Colpaert using a closely related procedure, i.e., the "shock-probe conflict" test (4). In this shock-probe test, the chamber floor is devoid of burying materials (e.g., bedding), and the index of fear is passive avoidance of the continuously electrified probe. Anxiolytic agents reliably increase the number of contact-induced shocks rats receive from the probe in a dose-dependent manner, while most nonanxiolytics do not produce this effect.

It should be apparent that the shock-probe test and the probe-burying test can be run concurrently (i.e., using a continuously electrified shock-probe in a chamber with bedding material). The potential advantage of this approach is that drug-induced decreases in probe-burying that are concurrent with increases in probe-contacts would provide convergent evidence of "anxiolytic" drug effects within a single setting.

In a preliminary study (5), the effects of chlordiazepoxide (2.5–10.0 mg/kg) and buspirone (0.05–1.0 mg/kg) were compared in this "shock-probe/burying" test. Both of these anxiolytic agents decreased the amount of time rats spent "burying" the continuously electrified (2 mA) shock-probe, and concurrently increased the number of contact-induced probe-shocks rats received. These bidirectional, anxiolytic drug effects increased as a function of drug dose, and were independent of changes in general activity. However, the drug-class specificity of these concurrent anxiolytic drug effects has not been adequately characterized. Thus, the purpose of the present study was to compare the effects of anxiolytic and nonanxiolytic agents in the shock-probe/burying test.

METHOD

The methods were basically the same as those described

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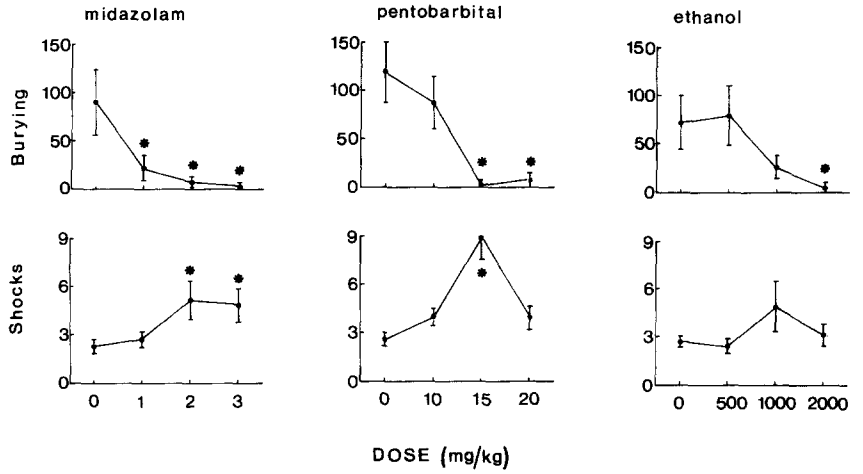

FIG. 1. Mean (SE) duration of probe burying (top panel) and mean (SE) frequency of probe-shocks (bottom panels) in rats injected with midazolam, pentobarbital, or ethanol.

previously [for further details see (5, 6, 8, 10)]. Two hundred and eighty naive, male (250–450 g) Sprague-Dawley rats served as subjects. The rats were individually housed for 3 to 7 days before the beginning of each test, and had unrestricted access to rat chow and water, under a 12-hr on/12-hr off light cycle (lights on at 7:00 hr). Testing occurred between 9:00 and 17:00 hr.

A separate testing room contained the $40 \times 30 \times 40$ cm Plexiglas test chamber. The chamber floor was evenly covered with 5 cm of odor-absorbent kitty litter. In the center of the front wall of the Plexiglas chamber, 2 cm above the bedding material, was a small hole through which a $6.5 \times 0.5 \times 6.5$ cm wire-wrapped probe could be inserted. Electric current was administered through two metal wires wrapped around the probe. Shock intensity was adjusted with a variable resistor in series with a 2000 V shock source and set at 2 mA. The behavior of each rat was recorded on video tape via closed circuit television.

The rats were habituated for 30 min on each of 4 consecutive days to the Plexiglas chamber, without the probe present, and then tested on day 5 with the proble inserted and continuously electrified at 2 mA.

Prior to being individually placed into the test chamber on the test day, the rats were randomized to groups ($n = 10$) and given intraperitoneal injections of either midazolam (0.0, 1.0, 2.0, or 3.0 mg/kg), pentobarbital (0.0, 10.0, 15.0, or 20.0 mg/kg), ethanol (0, 500, 1000, or 2000 mg/kg), scopolamine (0.0, 0.05, 0.25, or 1.25 mg/kg), chlorpromazine (0.0, 0.5, 2.5, or 5.0 mg/kg), yohimbine (0.0, 0.5, 1.0, or 2.0 mg/kg), or pentylenetetrazol (0.0, 5.0, 10.0, or 20.0 mg/kg). Drugs were dissolved in physiological saline (the "0" dose control) at a constant volume of 1 ml/kg, except for ethanol (injected as a 20% w/v solution). Thirty min later (or 10 min later for pentylenetetrazol), the rats were individually placed in the test chamber with the probe continuously electrified. Beginning with the first contact-induced probe shock, the rats' behavior was recorded for 15 min. The duration of probe-burying and the number of probe-shocks were measured by a "blind" observer. These data were analyzed with ANOVA, followed by pair-wise comparisons (t-tests, alpha $= 0.05$).

RESULTS

Figure 1 shows that both midazolam and pentobarbital suppressed probe-burying [midazolam: $F(3,36) = 4.81$, $p < 0.006$; pentobarbital: $F(3,36) = 6.65$, $p < 0.001$], and concurrently increased probe-shocks [midazolam: $F(3,36) = 3.45$, $p < 0.03$; pentobarbital: $F(3,36) = 14.25$, $p < 0.001$]. Ethanol produced a significant suppression of probe-burying, $F(3,36) = 2.91$, $p < 0.05$, but not a significant increase in probe-shocks, $F(3,36) = 1.80$, $p > 0.1$.

Although the nonanxiolytics scopolamine, chlorpromazine, yohimbine, and pentylenetetrazol each suppressed probe-burying to varying degrees (Fig. 2), these effects failed to reach the $p < 0.05$ level of significance [scopolamine: $F(3,36) = 0.86$, $p > 0.4$; chlorpromazine: $F(3,36) = 2.41$, $p > 0.08$; yohimbine: $F(3,36) = 0.94$, $p > 0.4$; pentylenetetrazol: $F(3,36) = 2.45$, $p > 0.07$]. None of these agents produced a significant increase in probe-shocks [scopolamine: $F(3,36) = 1.13$, $p > 0.3$; chlorpromazine: $F(3,36) = 1.00$, $p > 0.4$; yohimbine: $F(3,36) = 0.19$, $p > 0.5$], and pentylenetetrazol, a putative anxiogenic agent (6), produced a significant decrease in probe-shocks, $F(3,36) = 3.43$, $p < 0.03$.

DISCUSSION

The present results suggest that the shock-probe/burying test can distinguish anxiolytic from nonanxiolytic agents. Midazolam and pentobarbital each produced significant increases in contact-induced probe-shocks concurrent with significant decreases in probe-burying. These results extend those of a previous study showing similar effects for the anxiolytics chlordiazepoxide and buspirone (5). The effects of ethanol in the present study were similar but less dramatic than those of the benzodiazepine- or barbiturate-type anxiolytics. The nonanxiolytic agents scopolamine, chlorpromazine, yohimbine, and pentylenetetrazol did not produce significant, bidirectional effects on probe-shocks and probe-burying. The putative anxiogenic agent yohimbine was without effect in the present test. However, there was some suggestion of an anxiogenic effect for pentylenetetrazol, since it did produce a significant suppression of probe-shocks.

Why yohimbine was without significant anxiogenic effects in the present test, when prior experiments have shown it to increase probe-burying (11), is curious. One possibility is that prior tests, which used a single, 4-mA probe-shock, may have been more sensitive to the anxiogenic effects of yohimbine than the present test, which employed multiple, 2-mA probe-shocks. Thus, further studies are required in order to determine whether the shock-probe/burying test can reliably detect anxiogenic agents.

In summary, the shock-probe/burying test appears to provide convergent behavioral validation of anxiolytic drugs effects, since

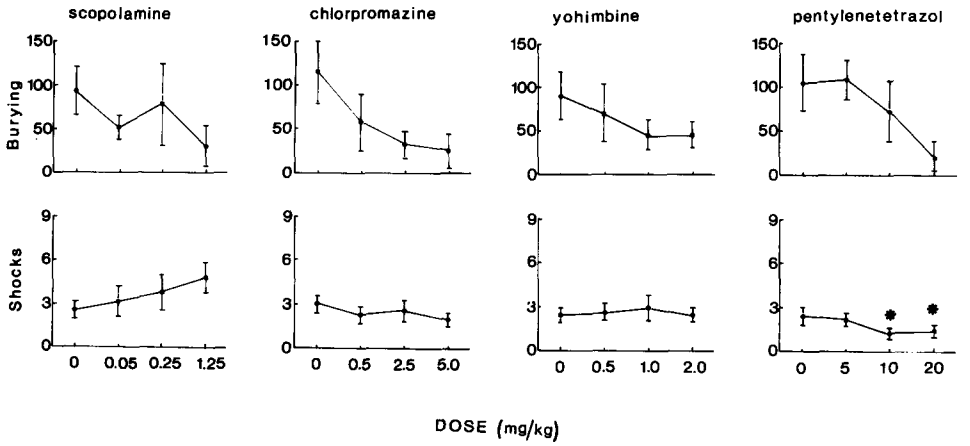

FIG. 2. Mean (SE) duration of probe-burying (top panel) and mean (SE) frequency of probe-shocks (bottom panels) in rats injected with scopolamine, chlorpromazine, yohimbine, or pentylenetetrazol. *$p<0.05$ compared to "0" dose control.

the number of contact-induced shocks is increased by anxiolytics at the same time as the amount of burying is decreased. Moreover, because these anxiolytic drug effects are bidirectional, they are difficult to explain in terms of general side-effects such as behavioral sedation [cf. (3,7)]. The test, therefore, offers advantages over those that employ only a single, unidirectional index of anxiolytic drug action, in that the effects of anxiolytics on probe-burying and probe-avoidance corroborate one another without requiring additonal tests in different settings. Further studies will be conducted to establish the neuropharmacological specificity of anxiolytic drug effects in the shock-probe/burying test, using specific antagonists of the GABA/benzodiazepine receptor complex (e.g., Ro 15-1788).

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