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RAPID COMMUNICATION

Discriminative Stimulus Properties of Midazolam Are Shared by a GABA-Receptor Positive Steroid

STEPHEN I. DEUTSCH*†[1] AND JOHN MASTROPAOLO*

**Psychiatry Service, Department of Veterans Affairs Medical Center, 50 Irving Street, N.W., Washington, DC 20422*
†Department of Psychiatry, Georgetown University School of Medicine, 3800 Reservoir Road, N.W., Washington, DC 20007

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DEUTSCH, S. I. AND J. MASTROPAOLO. *Discriminative stimulus properties of midazolam are shared by a GABA-receptor positive steroid.* PHARMACOL BIOCHEM BEHAV **46**(4) 963–965, 1993. – 3-Alpha-5-alpha-tetrahydrodeoxycorticosterone (alloTHDOC) is a 3-alpha ring A-reduced metabolite of deoxycorticosterone that has been shown to act via nongenomic mechanisms to modulate the $GABA_A$ receptor complex allosterically in vitro. Moreover, there are behavioral data consistent with the anxiolytic actions of GABA-receptor positive steroids. The drug discrimination paradigm was used in rats to demonstrate that effects of alloTHDOC are mediated by the $GABA_A$ receptor complex in the intact animal. In rats trained to discriminate 1.8 mg/kg of midazolam from saline, alloTHDOC substituted for the training stimulus.

$GABA_A$ receptor complex Drug discrimination Allotetrahydrodeoxycorticosterone

NATURALLY occurring 3-alpha-hydroxy ring A-reduced metabolites of progesterone and deoxycorticosterone represent a newly described class of endogenous allosteric modulator of GABA-gated chloride ion conductance [for review see (2)]. Binding to a site within the hydrophobic domain of the $GABA_A$-associated ionophore, these steroids influence both the affinity of the binding of ^{3}H-muscimol and ^{3}H-flunitrazepam, and inhibit the binding of ^{35}S-*t*-butylbicyclophosphorothionate to the $GABA_A$ receptor complex (3,5). Moreover, these steroid modulators influence the ability of GABA agonists to promote chloride ion uptake into synaptoneurosomes, a cell-free vesicular preparation containing both pre- and postsynaptic elements (5). These actions occur through nongenomic mechanisms at the cell membrane and are not mediated by classical cytosolic steroid receptors (3). The development of "GABA-receptor positive" steroids as medications for the treatment of anxiety, stress, sleep disturbances, and seizures requires demonstration of their efficacy in intact animal paradigms. For example, Crawley et al. (1) have shown that these steroids increase light/dark transitions, the number of times an animal will cross from a small dark area to a larger lighted area, and punished-licks made by animals in these two experimental paradigms predictive of anxiolytic actions. Drug discrimination has been used as a pharmacological tool to demonstrate that the actions of unknown compounds are mediated by specific neurotransmitter receptor complexes (4). Thus, the drug discrimination paradigm confirms the specific receptor-mediated basis of the GABA-receptor active steroid's action in the intact animal. In this study, drug discrimination was employed to demonstrate that 3-alpha-5-alpha-tetrahydrodeoxycorticosterone (allo-THDOC) shares discriminative stimulus properties with midazolam, a benzodiazepine agonist whose actions are mediated by the $GABA_A$ receptor complex.

METHOD

Subjects

Four experimentally naive, male Wistar Firth Rats, weighing between 200 and 250 g at the start of the experiment, served as subjects.

[1] Requests for reprints should be addressed to Stephen I. Deutsch, M.D., Ph.D., Chief, Psychiatry Service (116A), Department of Veterans Affairs Medical Center, 50 Irving Street, N.W., Washington, DC 20422.

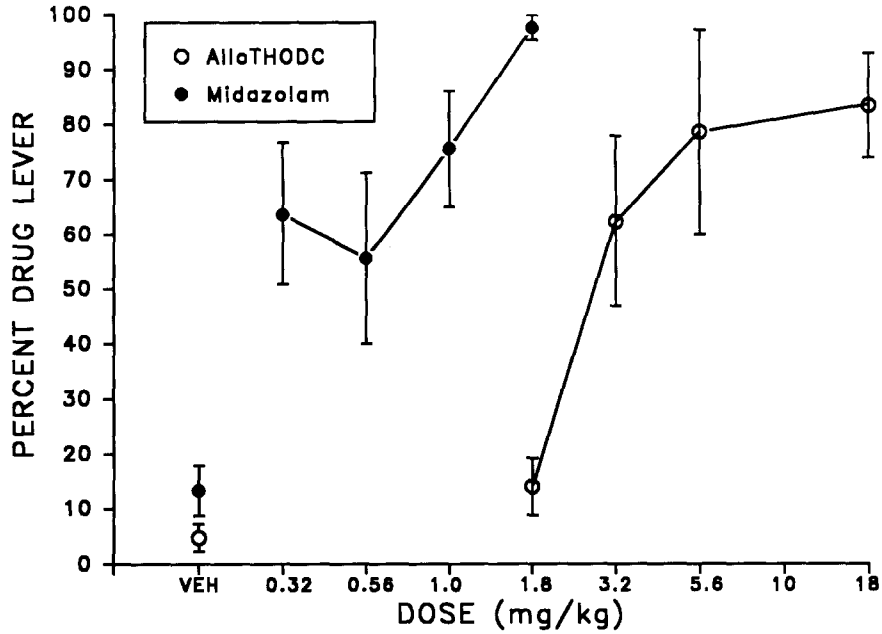

FIG. 1. This figure shows the mean (±SE) for substitution sessions with midazolam or its vehicle (●) and alloTHDOC or its vehicle (○).

Drugs

Midazolam maleate (generously donated by Hoffmann-La Roche, Inc., Nutley, NJ) was dissolved in distilled water. Allo-THDOC (Sigma, St. Louis) was dissolved in dimethyl sulfoxide (DMSO). All injections were IP in a volume of 0.1 ml/kg of body weight.

Procedure

The rats were reduced to 85% of their free-feeding body weights. Free access to water was always available in the home cage. Weight was maintained by food earned during experimental sessions and postsession feeding as necessary. Experimental sessions were conducted in eight identical operant chambers equipped with two response levers, two stimulus lights (one located above each lever), a house light, and a food-pellet dispenser (MED Associates, Lafayette, IN, model ENV 001). These chambers were enclosed in sound-attenuating chambers equipped with ventilation fans. All data collection and programmed contingencies were accomplished with an IBM compatible computer, OPN software (Operant Package for the Neurosciences, Fort Worth, TX) and a MED associates interface. In the drug discrimination, the rats were injected prior to each session with either 1.8 mg/kg of midazolam or its vehicle (distilled water). Animals were reinforced differentially, as a function of the injection prior to the start of the session. Specifically, following the injection of the 1.8 mg/kg of midazolam, the rats were required to complete the fixed ratio 10 (FR 10) on the right lever to obtain a food pellet (45 mg Noyes). Responses on the left lever were recorded, but had no programmed consequence. Following the injection of the drug vehicle, a FR 10 on the left lever was required to produce a food pellet, while responses on the right lever were recorded but had no programmed consequence. Animals were considered to have acquired the drug discrimination when the injection-appropriate lever was selected for completing the first FR on 8 out of 10 consecutive sessions. Once this training criterion was reached, substitution sessions began. Substitution sessions involved the injection of either a different dose of midazolam (0.32, 0.56, 1.0, 3.2, 5.6, or 10 mg/kg) or alloTHDOC (1.8, 3.2, 5.6, or 18

TABLE 1

THE MEAN (± SD) RATE OF RESPONDING (RESPONSES/S) DURING EACH OF THE TEST SESSIONS

Midazolam	Rate (responses/s)	AlloTHDOC	Rate (responses/s)
Vehicle	1.52 (± 0.62)	Vehicle	0.84 (± 0.43)
0.32	1.62 (± 1.34)	1.8	1.32 (± 0.44)
0.56	1.53 (± 0.74)	3.2	1.60 (± 1.17)
1	1.64 (± 0.64)	5.6	0.98 (± 0.80)
1.8	1.82 (± 0.89)	18	0.64 (± 0.89)

mg/kg). During substitution sessions, the completion of a FR10 on either lever ended the session and no food was delivered. Substitutions with varying doses of midazolam were conducted in a mixed order. Finally, substitutions of alloTHDOC were also completed in a mixed order.

RESULTS

As can be seen in Fig. 1, under training conditions responding was under the stimulus control of the drug injection. Specifically, approximately 98% of responses on the drug-appropriate lever followed an injection of 1.8 mg/kg of midazolam, whereas 13% of responses on the drug-appropriate lever followed an injection of the distilled water vehicle. Also, as expected, doses of midazolam lower than the training dose partially substituted for the training dose in a dose-dependent manner. Finally, it is clear that alloTHDOC produced a discriminative stimulus that substituted for midazolam. Although less potent on a mg/kg basis, at 18 mg/kg of alloTHDOC approximately 88% of responses were on the drug-appropriate lever.

DISCUSSION

The data show that alloTHDOC, a steroid that is a positive allosteric modulator of GABA-gated chloride ion conductance, produces stimulus properties similar to those of midazolam. The similarity of the subjective experience produced by alloTHDOC is consistent with its actions occurring at the $GABA_A$ receptor complex and suggests that it may function as an anxiolytic agent. The data also show that a GABA-receptor active steroid that is administered peripherally has potent central effects. The results lend strong support to the development of this class of endogenous modulator of the $GABA_A$ receptor complex as clinically useful psychopharmacologic agents. Future studies must address the relative advantages of GABA-receptor active steroids over existing classes of medications with regard to tolerance, dependence, and abuse liability.

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