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BRIEF COMMUNICATION

Comparison of the Discriminative Stimulus Function of Ethanol Following Intracranial and Systemic Administration: Evidence of a Central Mechanism

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HODGE, C. W. *Comparison of the discriminative stimulus function of ethanol following intracranial and systemic administration: Evidence of a central mechanism.* PHARMACOL BIOCHEM BEHAV 47(3) 743-747, 1994.—Rats were trained using a two-lever drug discrimination procedure to press one lever following systemic administration of ethanol (1.0 mg/kg, IP) and another lever following IP injections of saline. After determination of an ethanol generalization curve (0.25-1.25 g/kg, IP), rats were surgically implanted with bilateral stainless steel guide cannulae that terminated in the lateral ventricles. Following surgery, the generalization curve was redetermined and did not differ from presurgery values. Then, generalization to bilateral intracerebroventricular (ICV) injections of ethanol (600.0 and 900.0 mM, 1.0 μl/side) were administered alone and in combination with IP injections of ethanol. The ICV ethanol injections produced partial generalization, but the combination of ICV ethanol (600.0 and 900.0 mM) with IP ethanol (0.25 and 0.50 g/kg) injections were two- to threefold more potent then IP injections alone. Response rates were unaffected by any dose of ethanol tested. These data suggest central mediation of ethanol's discriminative stimulus function due to: 1) increased potency of systemically administered ethanol by centrally administered ethanol, and 2) partial generalization between centrally and peripherally administered ethanol.

Ethanol Drug discrimination Discriminative stimulus Intracerebroventricular injections Microinjection

THE subjective effects produced by drugs are thought to be influential in determining abuse potential (10,13). Drug discrimination procedures, which provide differential reinforcement for emitting a specific response in the presence of a drug, and for an alternative response in the presence of a vehicle, have been useful in characterizing the subjective effects of many drugs of abuse (2).

Recent studies utilizing direct brain injections have further clarified the neuropharmacology and neurophysiology of drug discrimination. For example, intracerebroventricularly (ICV) administered cocaine (23), midazolam (20), and opiates (8,15) generalize to peripherally administered training doses of the same drug. Further, microinjections of cocaine (22) and *d*-amphetamine (17) in the nucleus accumbens generalize to peripherally administered training doses. Midazolam microinjections in the amygdala antagonize the pentylenetetrazol discriminative stimulus (4). Thus, the nucleus accumbens and amygdala may represent sites of action for some of the subjective effects of psychomotor stimulants and benzodiazepines, respectively (4,17,22).

Ethanol can function as a discriminative stimulus (1-3,18). Neurochemical involvement in some of the discriminative stimulus properties of ethanol has been shown by stimulus generalization of drugs that act on receptor systems such as $GABA_A$ (2,7), and $5\text{-}HT_{1B}$ (21), but not dopamine D_1 or D_2 (21). Additionally, $5\text{-}HT_3$ antagonists block ethanol discriminative stimulus function at doses that also disrupt response rates (9). However, because systemically administered ethanol

distributes rapidly throughout all bodily tissue, the extent to which ethanol's discriminative stimulus functions result from action on specific central and/or peripheral receptor systems remains to be clarified.

The present study explored this premise using a two-lever drug discrimination procedure. As with other studies addressing the central mechanisms of drug discrimination (8,16, 20,23), the present study asked the initial question of whether centrally administered ethanol would generalize to peripherally administered ethanol. Rats were trained to press one lever following intraperitoneal (IP) injections of ethanol and another lever following vehicle injections. Subsequently, tests were conducted to determine if ICV-administered ethanol would generalize to IP-administered ethanol.

METHOD

Subjects

Male Long-Evans hooded rats ($N = 6$) obtained from the breeding facility at the University of Washington were housed individually in hanging stainless steel cages with food (Wayne Rodent Blox 8604, Wayne Laboratories, Bartonville, IL) always available. Water access was restricted to 1 h per day in the home cages with an additional 15-min access to a liquid sucrose (10% w/v) solution during experimental sessions. Initial body weights (mean grams ± SEM) increased from 291.4 ± 6.8 to 421 ± 15.6 throughout the experiment. The colony room was maintained on a 12 L : 12 D cycle with lights on at 0700 h. Temperature and humidity were maintained within NIH guidelines. All experimental sessions were conducted during the light portion of the cycle. All rats were experimentally and drug naive.

Apparatus

The apparatus used in this experiment has been described in detail previously (11). Briefly, experimental sessions were conducted in Plexiglas chambers (27 × 37 × 21 cm) located in sound-attenuating cubicles. The left side wall of each chamber was equipped with two liquid dispensers (Ralph Gerbrands Corp., model B-LH, Arlington, MA) that presented fluid in a 0.1-ml dipper for 3 s during each operation. Responses on levers located on the front and rear walls activated the left and right dippers, respectively. Exhaust fans masked external noise. Apple IIe microcomputers controlled experimental contingencies and recorded data. For ICV injections, stainless steel injection cannulae (33 ga) were connected with PE 20 tubing to two 1.0-μl syringes (Hamilton, Reno, NV) mounted on a microdrive pump (Harvard Apparatus, model 22).

Ethanol Discrimination Training and Testing

Rats were trained to press one lever following ethanol injections (1.0 g/kg, IP) and to press the other lever following saline vehicle injections under a fixed ratio 10 (FR10) schedule of sucrose (10% w/v) reinforcement. Injections occurred 10 min prior to the start of 15-min sessions. Following ethanol injections, completion of 10 responses on the appropriate lever produced sucrose. Responses on the inappropriate lever were recorded but produced no programmed consequences. Similarly, following saline injections, completion of each 10 responses on the saline-appropriate lever resulted in sucrose reinforcement, but responses on the ethanol-appropriate lever had no programmed consequences. There were an equal number of ethanol (E) and saline (S) training sessions that alternated on a daily basis (E, S, E, S . . .). Training sessions were conducted until performance in the E and S training sessions met the following criteria: the percentage of E- or S-appropriate lever press responses emitted prior to the first reinforcer, and during the entire session, exceeded 85% for 5 consecutive days.

After performance met the accuracy criteria, test sessions were conducted during which an ethanol (0.0–1.25 g/kg, IP) generalization curve was determined. Each rat received two injections of all ethanol doses. Test sessions were identical to training sessions except a) they were conducted in extinction (i.e., responses on both levers were recorded, but resulted in no reinforcement delivery), and b) novel doses of ethanol were administered. Test sessions were interspersed randomly with training sessions only if performance during training sessions continued to meet the accuracy criteria. If performance failed to meet the accuracy criteria, training was resumed until response accuracy was greater than 85% for 5 consecutive days. There was a minimum of two training sessions between test sessions.

Following completion of IP generalization testing, rats were surgically implanted with guide cannulae that terminated in the lateral ventricles. Operant sessions were not conducted for 1 week to allow recovery from surgery. After recovery, training sessions were resumed, until performance following IP injections again met the accuracy criteria. At this time, the IP generalization curve (0.0–1.0 g/kg) was redetermined to ascertain whether surgery influenced the ethanol discrimination. Subsequently, ICV generalization test sessions were conducted during which ethanol (600.0 and 900.0 mM, ICV) was administered in combination with ethanol (0.0, 0.25, and 0.50 g/kg, IP) and saline. This tested whether the two concentrations of ICV-administered ethanol would generalize to the IP training dose and whether ICV-administered ethanol would influence the discriminative stimulus function of IP-administered ethanol.

Surgery

Rats were anesthetized with Equithesin (3.0 ml/kg, IP) and placed in a stereotaxic device (David Kopf Instruments, model 1204 with rodent adaptor) with the incisor bar 3.3 mm below the horizontal plane. Stainless steel guide cannulae (26 ga) were implanted bilaterally to terminate in the lateral ventricle. Cannulae were secured to the skull with dental cement and stainless steel cranial screws. The guide cannulae were sealed with removable stylets (33 ga). The stereotaxic coordinates used for lateral ventricle were 8.0 mm from the interaural line, 1.4 mm lateral to the midline, and 2.2 mm ventral to the cortical surface (19).

Intracerebroventricular Injection Procedure

Two concentrations of ethanol (600.0 and 900.0 mM, ICV) were administered in combination with ethanol (0.25 or 0.5 g/kg, IP) or saline for a total of six ICV injections. Immediately prior to ICV injections, IP doses were administered and rats were placed in a plastic tub (30 cm in diameter by 14 cm deep) to minimize movement. Stylets were removed and the cannulae area was swabbed with sterile physiological saline. Bilateral artificial cerebrospinal fluid (ACSF) and ethanol injections (ICV) were performed through 33-ga stainless steel hypodermic tubing lowered to 1 mm below the end of guide cannulae. The pump delivered 1.0 μl/side/min. Injectors remained in place for 30 additional s to allow diffusion. New

sterile stylets were inserted after removal of the injectors. Operant sessions began 10 min after ICV injections.

Ethanol

Ethanol was diluted in 0.9% physiological saline for IP and ACSF for ICV injections. Ethanol (15% v/v) was administered in varied volumes (0.0, 0.25, 0.5, 1.0, and 1.25 g/kg, IP) to obtain, with the ethanol (1.0 g/kg) and saline training injections, a constant volume of 1.0 ml/kg. For ICV injections, ethanol (600.0 and 900.0 mM) was delivered bilaterally in a volume of 1.0 μl/side/min. All ethanol solutions were prepared immediately prior to each injection session.

Histology

Upon completion of the experiment, rats were deeply anesthetized with pentobarbital sodium and transcardially perfused with a sodium phosphate buffer solution (pH 7.5) followed by 10% formaldehyde. Brains were removed and stored in 10% formaldehyde for 7 days, after which they were cut into 60-μm sections and stained with cresyl violet. Placement of cannulae was verified using a standard light microscope (Bausch and Lomb, Galen III). Only the data resulting from bilateral lateral ventricle injections were used in the analysis.

Data Analysis

The number of responses on each lever were expressed as a percentage of total lever presses a) prior to delivery of the first reinforcer, and b) for total session responses. Data were plotted as percentage of responses on the ethanol-appropriate lever. Response rates were expressed as number of responses/s and analyzed only for the period up to a) delivery of the first reinforcer during training sessions, or b) completion of 10 responses on either lever during generalization test sessions. Response rates were not analyzed for total session duration because responding terminated rapidly following completion of the first 10 responses during test sessions that were conducted in extinction. Data from ICV injection test sessions were compared to data from postsurgery IP injection sessions by paired *t*-test.

RESULTS

Approximately 40 training sessions were required for performance to meet criteria. Subsequently, stimulus control by the ethanol (1.0 g/kg) and saline IP injections maintained response accuracy above 90% and no supplemental training sessions were required. Data from two rats were excluded from analysis for the following reasons: death during initial phase (n = 1), and failure to meet accuracy criteria after 60 sessions (n = 1). Thus, data are presented for four rats.

IP Injections

Figure 1 shows generalization curves from pre- and postsurgery ethanol (IP) injection sessions and postsurgery ICV ethanol injection sessions. The pre- and postsurgery IP generalization curves were not statistically different, indicating that the surgical procedure had no effect on ethanol discrimination. Pre- and postsurgery ED_{50}s for responses emitted prior to the first reinforcer were: IP presurgery = 0.6 g/kg, IP postsurgery = 0.52 g/kg (Fig. 1, top). The ED_{50}s for total session responses were: IP presurgery = 0.45 g/kg, IP postsurgery = 0.45 g/kg (Fig. 1, bottom).

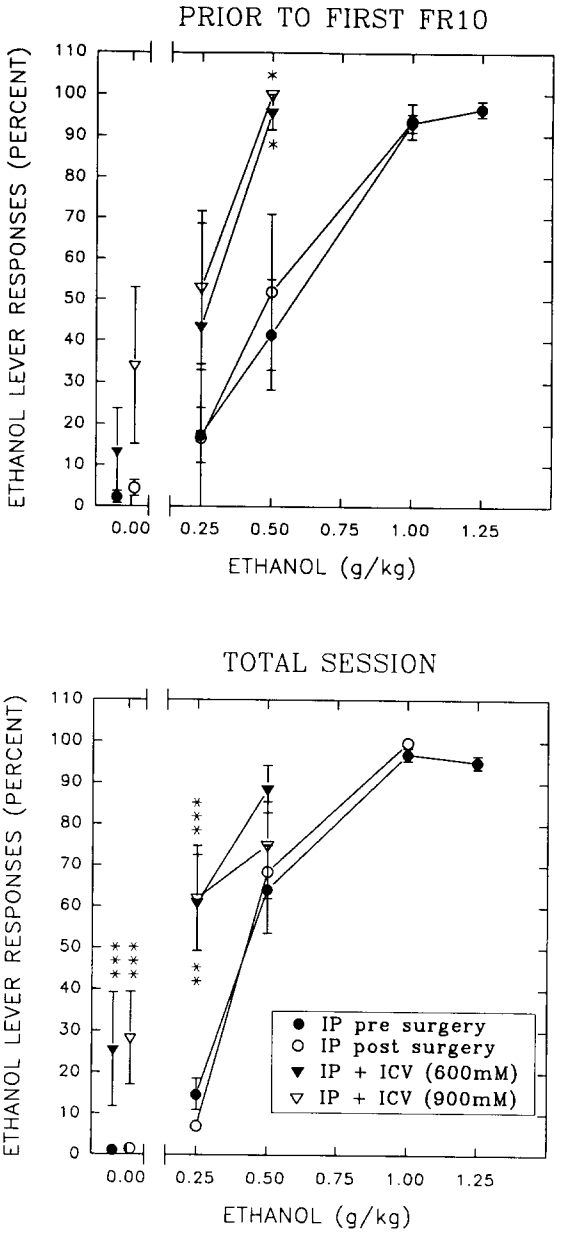

FIG. 1. Mean percentage of ethanol-lever responses in test sessions prior to completion of the first 10 responses on either lever (top) and for the total session (bottom) after ethanol injections by IP and ICV routes of administration. Data points represent two injections per rat for IP administration and one injection per rat for ICV administration. Overlapping data points at the 0.0 dose were displaced horizontally for visual clarity. Error bars are ± SEM. Asterisks indicate significantly different from corresponding postsurgery control value: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (paired *t*-test).

ICV Injections

The ICV ethanol injections in combination with saline vehicle IP injections resulted in partial generalization on both response measures (Fig. 1). The combination of ICV ethanol (600.0 and 900.0 mM) and IP ethanol (0.25 and 0.5 g/kg) shifted the generalization curves to the left. The ED_{50}s, prior

to the first FR10, for combined (IP + ICV) injections were: 600 mM = 0.28 g/kg, and 900 mM = 0.22 g/kg (Fig. 1, top). Maximal generalization (90–100%) occurred at 0.5 g/kg (a twofold shift to the left of the training dose) for ethanol-lever response percentages prior to the first reinforcer. Total session responding ED_{50}s for combined (IP + ICV) were: 600 mM = 0.14 g/kg, and 900 mM = 0.16 g/kg. Thus, the ED_{50}s following both doses of ICV ethanol were shifted approximately twofold to the left of the ED_{50}s generated following post-surgery IP injections. Maximal generalization, as measured by total session responding, was somewhat lower because tests were conducted in extinction.

No decrements in response rates prior to completion of the first FR10 (data not shown) were observed at any dose of ethanol tested, suggesting that motor inhibition did not influence responding.

DISCUSSION

A number of centrally administered drugs that act on specific receptor systems have been shown to function as discriminative stimuli. For example, ICV-administered midazolam (20) and cocaine (23) generalize to IP training doses of the same drug. Centrally administered morphine (16), fentanyl (6), and pentylenetetrazol (4) have also been shown to serve discriminative stimulus functions. The data from the present experiment extend these findings to include the discriminative stimulus effects of centrally administered ethanol.

Following discrimination training with ethanol (1.0 g/kg, IP), administration of test doses of ethanol (0.0–1.25 g/kg, IP) resulted in a dose-dependent increase in responding on the ethanol-appropriate lever. Both doses of ICV-administered ethanol potentiated the dose-dependent increase in the percentage of ethanol-lever responses produced by IP-administered ethanol by approximately twofold. Complete generalization to the training dose of ethanol (1.0 g/kg, IP) occurred at 0.5 g/kg in combination with both doses of ICV ethanol. This suggests that the small volume of ICV-administered ethanol acted directly on CNS mechanisms to increase the potency of the IP ethanol discriminative stimulus.

Neither dosage of ethanol administered centrally alone produced complete generalization to peripherally administered ethanol, but both ICV doses resulted in partial generalization. Partial generalization suggests a number of possible explanations. Intermediate levels of drug-lever responding following lower doses of the training drug could indicate random or chance responding, an artifact of the testing conditions, or a reliable measure of the similarity of the subjective effects between the training and testing doses (12). The first two possibilities are difficult to rule out in any drug discrimination experiment. However, the present data show a dose-related increase in partial generalization as measured by drug-lever responding prior to completion of the first 10 responses (Fig. 1, top). This suggests that the partial generalization represented a quantitative variation in the stimulus dimension generated by the doses of ICV- and the training dose of IP-administered ethanol rather than random responding. Thus, partial generalization of the ICV ethanol may have been a function of the concentration (600.0 and 900.0 mM) or volume (1.0 μl/side) injected, or rapid dilution of ethanol in CSF.

The present data do not address specific CNS sites that may mediate ethanol's discriminative stimulus function. However, drugs that act on numerous receptor systems, such as GABA (2,7), 5-HT_{1B} (21), and 5-HT_3 (9), have been shown to influence ethanol discrimination. Administration of ethanol has been shown to produce dose-related increases in dopamine in the nucleus accumbens (14) that are blocked by the 5-HT_3 antagonist ICS 205-930 (5), suggesting that blockade of the ethanol discriminative stimulus may be mediated by 5-HT_3 mechanisms in the nucleus accumbens (9). Thus, future studies of the CNS mechanisms of ethanol discrimination could incorporate site-specific brain injections of ethanol or drugs shown to influence ethanol discrimination when administered systemically.

This approach has been utilized in studying the CNS mechanisms of drugs acting on specific receptor systems. Direct injections of psychomotor stimulants, such as *d*-amphetamine (17) and cocaine (22), into the dopamine-rich area of the nucleus accumbens generalize to systemically administered doses. Midazolam (2.0–32.0 μg) injected in the amygdala produced a dose-dependent antagonism of the pentylenetetrazol (20 mg/kg, IP) discriminative stimulus (4). Morphine injected in the periaqueductal gray generalizes to systemically administered morphine (16). These data suggest specific central sites of action for the discriminative stimulus functions of these drugs and may prove useful in further characterizing the mechanisms that control ethanol discrimination.

Central mediation of ethanol's discriminative function is suggested from the present data by: 1) increased potency of systemically administered ethanol when combined with centrally administered ethanol, and 2) partial generalization by centrally administered ethanol to systemically administered ethanol. Future research, incorporating CNS site-specific microinjections of drugs acting on specific receptor systems, could elucidate the neurochemical and neuroanatomical mechanisms involved in the central regulation of ethanol's discriminative stimulus function. Understanding the neurobiological factors involved in ethanol discrimination may contribute to pharmacotherapeutic interventions in alcohol abuse.

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