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Cholecystokinin as a Stimulus in Drug Discrimination Learning

PAMELA M. MELTON,[1] JEFFREY A. KOPMAN AND ANTHONY L. RILEY

Psychopharmacology Laboratory, Department of Psychology, The American University, Washington, DC 20016-8062

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MELTON, P. M., J. A. KOPMAN AND A. L. RILEY. *Cholecystokinin as s stimulus in drug discrimination learning.* PHARMACOL BIOCHEM BEHAV 44(2) 249–252, 1993.—Animals were trained to discriminate a relatively low dose of the octapeptide cholecystokinin (CCK) from distilled water within the conditioned taste aversion baseline of drug discrimination learning. Specifically, rats were injected with CCK (5.6 μg/kg) prior to the presentation of saccharin-LiCl pairings and with the CCK vehicle prior to the presentation of saccharin alone. After 10 conditioning trials (40 days), subjects acquired the discrimination, avoiding saccharin consumption following administration of CCK and consuming the same saccharin solution following the drug vehicle. Once the discrimination was acquired, a generalization function was determined for doses above and below that of the training stimulus. At doses below the training dose of CCK (i.e., 0, 3.2, and 4.2 μg/kg), subjects drank at control levels, whereas at the training dose and above (10 μg/kg) subjects significantly reduced consumption. That a relatively low dose of CCK can be used as a discriminative stimulus within a drug discrimination design may be important in that the procedure can now be used in the assessment of the pharmacological characteristics of CCK at a dose similar to that used in other behavioral assessments of the compound.

Cholecystokinin Drug discrimination learning Conditioned taste aversions Generalization

DE WITTE and colleagues (5) recently reported the acquisition of drug discrimination learning [see (9)] using the sulfated form of the octapeptide cholecystokinin (CCK) as the training stimulus. Specifically, rats were reinforced with electrical brain stimulation for responding [fixed ratio (FR) 10] on a specific lever following injection of 20 μg/kg CCK and on a different lever following administration of the CCK vehicle. After 38 CCK trials (a total of 105 days), six animals acquired discriminative control, that is, meeting the criterion of 82% CCK-appropriate responding on at least 8 of 10 consecutive sessions. Although CCK was effective as a drug stimulus, it should be noted that the dose used to establish the discrimination (i.e., 20 μg/kg) was high and considerably outside the range used in other assessments of the effects of CCK, for example, satiety (7), conditioned flavor preferences (15), suppression of activity (3), suppression of ethanol intake (11), changes in taste responsiveness (8), and antagonism of opiate-mediated responses (6). Given that the effects of CCK within a number of procedures have been reported to be dose dependent (1), it is unknown if and to what extent the results from the drug discrimination procedure utilizing a 20-μg/kg dose of CCK would generalize to these other behavioral baselines assessing the effects of CCK at lower doses.

Recently, our lab and others reported the rapid acquisition of drug discrimination learning within the conditioned taste aversion baseline of drug discrimination learning. For example, Mastropaolo et al. (12) demonstrated that animals injected with phencyclidine (PCP) prior to saccharin-LiCl pairings and the PCP vehicle prior to saccharin alone acquired the discrimination in as few as three trials, avoiding saccharin when it was preceded by PCP and drinking the same saccharin solution when it was preceded by distilled water [for a review, see (16)]. The sensitivity of the taste aversion baseline has also been demonstrated in the fact that naloxone, a drug heretofore ineffective as a discriminative cue (14), was effective as such a cue in this design (10). Given the relative sensitivity of the taste aversion baseline of drug discrimination learning, it is possible that discriminative control with CCK might be established within this design at a dose that better approximates those used in other assessments of the behavioral effects of CCK. To that end, in the present experiment drug discrimination learning was assessed with a moderate dose of CCK (5.6 μg/kg) using the taste aversion procedure.

METHOD

Subjects

Subjects were 24 drug-naive, female rats of Long-Evans descent, approximately 270–310 g at the start of the experiment. They were housed in individual wire mesh cages and

[1] To whom requests for reprints should be addressed.

maintained on a 12 L : 12 D cycle and at an ambient temperature of 23°C for the duration of the experiment. Standard rat chow was available ad lib.

Drugs

The sulfated form of cholecystokinin octapeptide (generously supplied by the Squibb Institute) was prepared in distilled water in a concentration of 10 μg/ml and injected in a volume of 0.56 ml/kg.

Procedure

Phase 1: Acquisition. During the light phase (0800–1000 h), subjects were given restricted access to water for 30 consecutive days. Over this period, the duration of restricted access decreased from 20 min (days 1–13) to 10 min (days 14–22) to the terminal value of 5 min (days 23–30). On days 31–33, a novel saccharin solution (0.1% w/v saccharin sodium salt, Sigma Chemical Co., St. Louis, MO) replaced water during the 5-min access period (saccharin habituation) and was preceded on the last 2 days of saccharin habituation by an IP injection of distilled water (0.56 ml/kg). On day 34, all subjects were given an IP injection of 5.6 μg/kg CCK 5 min prior to 5-min saccharin access. Immediately following saccharin access, subjects were matched on saccharin consumption and assigned to one of two groups (groups L and W, n = 12 per group). Subjects in group L were given an IP injection of 1.8 mEq/0.15 M LiCl (76.8 mg/kg), while subjects in group W were given an equivolume injection of the distilled water vehicle. On the following 3 days, all subjects were injected with distilled water 5 min prior to saccharin access. No injections were given following saccharin access on these recovery days. This alternating procedure of conditioning (CCK–saccharin–LiCl or CCK–saccharin–distilled water) and recovery (distilled water–saccharin) was repeated until discriminative control had been established for all subjects (i.e., each subject had consumed at least 50% less than the mean of control subjects on three consecutive conditioning trials).

Phase 2: Generalization. Following acquisition of the discrimination, CCK dose-response functions were determined for six animals from each group. The procedure during this phase was identical with that described above with the following exception: On the second recovery day following each conditioning trial, subjects were injected with one of a range of doses of CCK (0, 3.2, 4.2, 5.6, and 10 μg/kg, all at a concentration of 10 μg/ml) 5 min prior to saccharin access. No injections followed saccharin access on these probe sessions. On any specific probe day, a subject was given a CCK injection only if it had consumed at least 50% less than the *mean* of control subjects on the immediately preceding conditioning trial. Doses were administered in a mixed pattern with all subjects receiving each dose at least once. A dose was tested a second time only if there was extreme intergroup variability. Under such conditions, the average of the two administrations was used in graphic representation and statistical analysis. To maintain body weight during generalization testing, all subjects were given 5-min additional access to saccharin approximately 6 h after CCK injections on conditioning and probe days.

Statistical Analysis

A two-tailed Mann-Whitney U-test was preformed on all between-group comparisons of saccharin consumption. A two-tailed Wilcoxon matched-pairs signed-ranks test (Z) was performed on all within-group comparisons of saccharin consumption over repeated trials. Absolute probabilities are presented for all comparisons.

RESULTS

Phase 1: Acquisition

Figure 1 presents the mean amount (±SEM) of saccharin consumed for groups L and W during saccharin habituation and over 12 repeated conditioning/recovery cycles. As illustrated, there was no significant difference in saccharin consumption between groups L and W during saccharin habituation (U = 60, 84, p = 0.49). The mean consumption of saccharin averaged over the 3 days of saccharin habituation was 10.3 and 10.5 ml for subjects in groups L and W, respectively. On the initial conditioning trial, subjects in both groups L and W significantly decreased saccharin consumption below habituation levels (Z = 2.55 and 2.36, p = 0.01 and 0.018, respectively). There were no significant differences between groups on this initial conditioning trial (U = 71, 73, p = 0.56). The groups did differ in saccharin consumption on the third conditioning trial, at which point subjects in group L drank significantly less than subjects in group W (U = 39, 105, p = 0.054). This difference was maintained for the remainder of conditioning. On the final conditioning trial of this phase, subjects in groups L and W drank 2.0 and 8.8 ml, respectively (U = 2, 142, p = 0.01). During recovery sessions, saccharin consumption for both groups remained high, approximating habituation levels.

Phase 2: Generalization

Figure 2 illustrates the mean amount (±SEM) of saccharin consumed for subjects in groups L and W following various doses of CCK. It should be noted that generalization functions were determined in only five of the six experimental subjects as one subject did not maintain discriminative control. As illustrated, consumption of saccharin for subjects in group W did not vary systematically over the various doses of CCK. For subjects in group L, there was an inverse relationship between the dose of CCK and amount of saccharin consumed. At the lower doses of CCK (i.e., 0, 3.2, and 4.2 μg/kg), consumption for subjects in group L did not differ from that for subjects in group W (U = 9.5, 20.5, p = 0.32; U = 5.5, 24.5, p = 0.08; and U = 5, 25, p = 0.06, respectively). At the training dose of CCK (5.6 μg/kg), consumption was markedly reduced (to a mean of 1.25 ml). At this dose, subjects in group L drank significantly less than subjects in group W (U = 0, 25, p = 0.01). Consumption for subjects in group L remained low at the 10-μg/kg dose of CCK and significantly below that for subjects in group W (U = 0, 30, p = 0.01). Although consumption at this dose appeared to increase above the level of the training dose, this increase was a function of a single animal that drank at control levels on one determination at this dose. At the second determination, this animal drank 0 ml.

DISCUSSION

Animals injected with CCK prior to presentation of a saccharin–LiCl pairing and with the CCK vehicle prior to presentation of saccharin alone rapidly acquired the CCK/vehicle discrimination. That CCK is an effective drug stimulus within

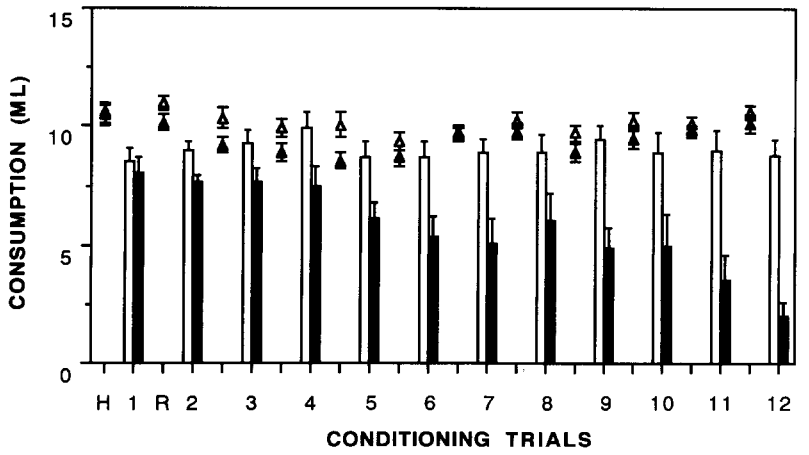

FIG. 1. Mean amount (±SEM) of saccharin consumed for subjects in groups L and W over repeated conditioning trials (filled and open columns, respectively). The filled and open triangles represent mean saccharin consumption (±SEM) for subjects in groups L and W, respectively, averaged over the 3 days of saccharin habituation (H) and over the three recovery sessions (R) between each conditioning trial.

the taste aversion baseline is consistent with prior work utilizing this procedure in the assessment of drug discrimination learning (12). Further, the acquisition of discriminative control with a dose of 5.6 μg/kg CCK replicates and extends the earlier work by De Witte and colleagues (5), who reported discriminative control with a higher dose of CCK (20 μg/kg). Direct comparisons between the two procedures in terms of the speed of acquisition are difficult given that the two discrimination procedures differ along numerous dimensions. The time required to establish discriminative control within the aversion design (based upon individual subjects meeting a preset criteria for the initiation of generalization testing), however, appears to be less than that for the brain stimulation-maintained discriminated operant design (40 vs. 105 days, respectively) even though the dose used was approximately four times less. This difference in the rapidity with which the discrimination is acquired is consistent with other work with the aversion design reporting the relative rapid acquisition of discriminative control (10,12).

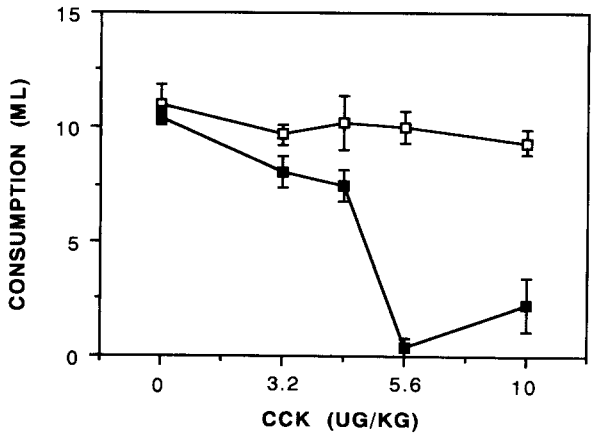

FIG. 2. Mean amount (±SEM) of saccharin consumed for subjects in groups L (■) and W (□) following various doses of cholecystokinin (CCK) (0.0–10.0 μg/kg) during generalization testing.

In the present procedure, animals were injected with CCK 5 min prior to 5-min access to saccharin and, as noted, CCK control was established within 10 conditioning trials. Interestingly, in unpublished work from our lab other temporal parameters did not support discriminative control with CCK. Specifically, when CCK was administered either 15 or 10 min prior to 20-min access to saccharin there was only marginal evidence of discriminative control. When CCK was administered 10 min prior to 10-min access, control was similarly not acquired. It is not clear what underlies the failure of CCK to establish control under these specific parameters, although given the short half-life of CCK (2) it is possible that a testing situation greater than 10 min in duration results in a loss of or change in CCK activity (and its stimulus properties). Such changes in stimulus control have been reported with other drugs (albeit over longer time periods) when temporal analyses of stimulus control have been assessed (4).

During generalization tests with various doses of CCK, consumption decreased with increasing doses. This inverse dose-response function (i.e., an increase in drug-appropriate responding with increasing doses of the drug) is similar to that reported for other compounds both within the taste aversion baseline of drug discrimination learning (10,12,16) as well as more traditional assessments of stimulus control by drugs (13). Interestingly, the effects of the 4.2-μg/kg dose were variable (either vehicle- or drug-appropriate responding), suggesting that this dose was near the threshold for stimulus control. Such "all or none" responding has been reported to be characteristic of stimulus control of drug stimuli within most drug discrimination designs [(13); but see (17)]. Interestingly, within the taste aversion procedure, responding during generalization tests is often more graded, with intermediate doses producing intermediate suppression of consumption (16). The basis for the more quantal dose-response function for CCK within the taste aversion design remains unknown.

The present demonstration of discriminative control by a

relatively low dose of CCK suggests that the taste aversion baseline of drug discrimination learning may be a useful procedure to assess other characteristics of CCK, for example, its similarities to other compounds (gut peptides, opiate antagonists, emetics, adipsogenics), what classes of compounds might serve as antagonists or agonists to its effects, and what specific receptor(s) might mediate its stimulus properties. Similar characteristics for other drugs serving stimulus functions have been well documented using the drug discrimination procedure, and given the apparent similarities of the stimulus effects reported with CCK to those of other compounds CCK is likely to be subject to similar assessments.

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