

Chlordiazepoxide Directly Enhances Positive Ingestive Reactions in Rats

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Received 19 February 1985

BERRIDGE, K C AND D TREIT *Chlordiazepoxide directly enhances positive ingestive reactions in rats* PHARMACOL BIOCHEM BEHAV 24(2) 217-221, 1986 —Benzodiazepines such as chlordiazepoxide (CDP) promote feeding in a number of species. This effect has been interpreted generally to be an indirect consequence of benzodiazepine anti-anxiety action, although some have questioned whether it might not reflect instead a direct action upon the reinforcing properties of foods. The present study employed a behavioral measure that can discriminate between these possibilities: palatability-dependent consummatory actions elicited in rats by tastes. The results suggest that chlordiazepoxide enhances the positive palatability of tastes selectively while having little or no effect on aversive palatability. The net effect is to make tastes more reinforcing following CDP administration.

Chlordiazepoxide	Palatability	Benzodiazepines	Anxiolytic	Ingestion
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ONE of the first documented effects of anxiolytic agents such as the benzodiazepines was their ability to increase the ingestion of food and water by rats and dogs (e.g., [39]). Stimulatory effects on food and fluid intake have since been consistently replicated in both satiated (e.g., [14, 36, 48]) and deprived animals (e.g., [14, 40, 44]) with both novel (e.g., [42]) and familiar food (e.g., [12, 50]) or fluids (e.g., [17, 44]), in novel (e.g., [10, 41]) and familiar environments [43, 49], and in a variety of species, including rats (e.g., [18, 34]), hamsters [9], cats [23, 27, 35], dogs (e.g., [23]), pigeons [20], horses [11], rhesus monkeys [26] and humans [2, 45]. Thus it is not surprising that drug-induced increases in ingestion under conditions of novelty (e.g., [10]) or punishment (e.g., [48]) have been taken as simple indices of anxiolytic drug action (e.g., [10, 41]).

The facilitatory effects of anxiolytics on food and fluid consumption have traditionally been interpreted in terms of a drug-induced reduction in the aversive components of the test (e.g., novelty, shock-induced conflict), which otherwise suppress consumption (e.g., [10]). While this interpretation is plausible to the extent that compounds such as the benzodiazepines do have well documented anti-anxiety effects in humans (e.g., [29]), it has also been argued that anxiolytics might enhance ingestive appetitive behaviors and consumption through a direct stimulatory effect on the biological mechanisms controlling food and fluid intake (e.g., [15, 16, 46, 49]). The empirical support for these alternative views is complex [1, 12, 15, 16, 26, 36, 40, 49], and often contradictory (e.g., [19] vs [42]), but without a successful resolution of this controversy it is difficult to interpret the hyperphagic and hyperdipsic effects of benzodiazepines, and equally difficult to use measures of ingestion confidently as measures of the anti-anxiety mechanisms of drugs (cf

[47, 49]). Methodologies employing simple intake measures of ingestion are unable to resolve this controversy, since intake measures are expected to be affected equally by anti-anxiety and by food-reinforcing actions of any drug.

A useful method for assessing specific changes in palatability is provided by the ingestive and aversive consummatory actions that are elicited in rats by infusion of solutions into the mouth [31]. These actions are highly sensitive to the palatability of the infused solution [30]. A response profile can be constructed for a given taste by counting the incidence of each ingestive and aversive action elicited by that taste. When this is done for naive rats the profiles obtained for tastes of differing preference (e.g., glucose, sodium chloride, ammonium chloride, and quinine hydrochloride) parallel intake measures closely [7]. These species-typical consummatory actions are affected appropriately by the same physiological state variables that shift human ratings of palatability, such as caloric satiety or sodium balance [4, 8, 13, 32]. They are also affected by those conditioned associations between tastes and postingestive consequences that influence human palatability ratings, such as conditioned taste aversions [7, 28, 32, 37]. Equally important for the purposes of this study, these actions appear generally *not* to be affected by conditioned factors that would *not* change human palatability ratings [38]: for example, associations of the taste of sucrose with shock or with lactose intolerance do *not* shift the actions elicited by sucrose from ingestive to aversive, even though the associations do suppress sucrose intake (and may terminate prematurely a purely ingestive sequence [37]).

The specific control of these responses by palatability provides a way of dissecting the psychological nature of the hyperphagic effect of CDP administration. Diffusely aver-

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sive states that suppress food intake, such as anxiety or fear induced by footshock pairing, either do not change consummatory actions elicited by tastes [37] or else suppress equally both positive and aversive actions (suggested by the premature termination of drinking of a shock-paired taste, without a shift in the *proportion* of ingestive and aversive actions, reported by Pelchat and colleagues [37] and supported by personal observations) These consummatory actions may thus provide a more accurate index of CDP effects on palatability than intake measures If the stimulatory effects of CDP upon feeding are due entirely to its anti-anxiety action, then the administration of a CDP dose that reliably increases intake would be expected either to leave infusion-elicited actions unchanged or to enhance equally both ingestive and aversive actions On the other hand, if the effect of CDP upon intake depends directly upon appetite or palatability-related reinforcing mechanisms, then the administration of a CDP dose that increases intake would be expected to bias the consummatory action profile toward positive ingestive responses

METHOD

Animals

Eight naive male Sprague-Dawley rats (250–300 g) were housed in groups of 2–3 throughout the experiment and allowed ad lib access to food and water To allow taste solutions to be infused into the mouth, each rat was anesthetized with ketamine (10 mg/kg) and acepromazine (1 mg/kg) and implanted with two permanent oral cannulae [31] These bilateral cannulae enter the mouth lateral to the first maxillary molar and exit the head at the dorsal skull, where they are anchored with skull screws and acrylic cement The cannulae provide channels into which fine tubing (PE 10) can be fitted, permitting the infusion of solutions [31]

Taste Stimuli and CDP Dosage

Three different taste stimuli were used 0.03 M sucrose, which elicits ingestive actions primarily, 0.01 M HCl (mildly sour to humans), which elicits both ingestive and aversive actions, and 3×10^{-5} M quinine HCl, which elicits aversive actions primarily All concentrations were chosen to be the lowest that would reliably elicit taste-appropriate actions Low concentrations were used in order that any behavioral facilitation effects of CDP would not be obscured by ceiling effects

Each rat received one stimulus presentation trial per day Each taste stimulus was tested twice once 30 min after the rats received CDP (10 mg/kg, IP) dissolved in isotonic saline (1 ml/kg), and once after receiving an equal volume of saline alone These trials were run in counterbalanced order The CDP dose was chosen on the basis of previous reports showing it to be within the range that reliably promotes feeding-related behavior in rats (e.g., [14, 16, 18, 19, 20]) Prior to testing, each rat received three CDP injections (one per day) to induce tolerance to initial sedative effects [24]

Apparatus

At testing, a rat's cannulae were connected to a stimulus delivery tube, and the rat was allowed to habituate for 5 min to the clear plastic test chamber A 1 ml volume of the taste solution was then infused into the mouth at a constant rate over 1 min Each rat was habituated to this procedure on two

consecutive days prior to testing, using distilled water as the taste stimulus The behavior of a rat was videotaped during testing via a mirror mounted beneath the transparent floor of the test chamber

Behavioral Criteria

Each rat was scored for the occurrence of ingestive and aversive actions Strongly ingestive actions are paw licking, lateral tongue protrusions (nonrhythmic) past the lip followed by forward extension, lasting 164 ± 9 msec (mean \pm SEM), and rhythmic tongue protrusions along the midline, with a cycle length of 166 ± 7 msec A weakly ingestive or neutral action is rhythmic mouth movement without tongue protrusion at the same or lower frequency as rhythmic tongue protrusion A behavior scored as neutral or weakly aversive is passive drip, the passive leaking of fluid from the mouth Strongly aversive actions are gapes (large openings of the mandible and retraction of the lower lips lasting 124 ± 8 msec), chin rubbing (bringing the mouth in direct contact with the floor and projecting the body forward), face washing (either a single wipe with the paws or a bout of several wipes), forelimb flails (shaking of the forelimb with a frequency of greater than 60 Hz), headshaking (at greater than 60 Hz), paw treading (planting of the forelimbs on the floor and alternating forceful strokes forward and back), and rapid locomotion about the chamber (cf. [30,31] for further information on the classification of these actions)

Data Analysis

Videotapes were scored at 1/10 speed by an observer blind to the drug condition of the rats For the purpose of quantifying the number of responses emitted, discrete actions such as lateral tongue protrusions, gapes, chin rubs, and bouts of face washing, forelimb flailing, headshakes, paw treading, and locomotion were recorded each time they occurred Continuous actions that typically persist for relatively long periods were recorded as follows: paw licks, mouth movements, and passive dripping were recorded in 5-sec bins (any occurrence of these behaviors up to 5 sec duration was counted as a single occurrence) Rhythmic tongue protrusions were scored in the same way in 2-sec bins [6]

RESULTS

CDP increased the number of ingestive actions elicited by all tastes while either suppressing or leaving unchanged the number of aversive actions (Fig. 1) For sucrose, significantly more ingestive rhythmic tongue protrusions ($p < 0.02$, Wilcoxon matched-pairs test), lateral tongue protrusions ($p < 0.02$), and ingestive actions combined (i.e., paw licks, rhythmic tongue protrusions, and lateral tongue protrusions added together, $p < 0.01$), were elicited after CDP than after saline injections Aversive actions were not affected significantly, either individually or combined For HCl, ingestive rhythmic tongue protrusions ($p < 0.02$) and lateral tongue protrusions ($p < 0.02$) were again increased by CDP, as were ingestive actions combined ($p < 0.01$) Aversive forelimb flails to HCl were reduced by CDP ($p < 0.05$), as were aversive actions combined ($p < 0.05$) For quinine, ingestive rhythmic tongue protrusions ($p < 0.05$), lateral tongue protrusions ($p < 0.02$), and ingestive actions combined ($p < 0.02$) were increased by CDP, while aversive actions were unchanged either individually or combined

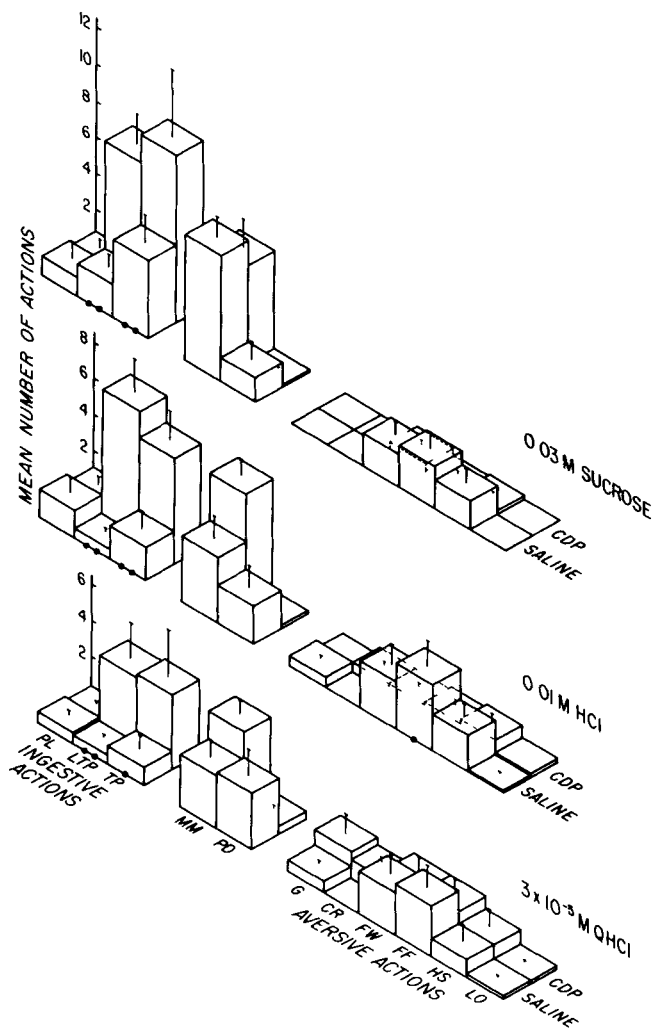


FIG 1 Taste-elicited actions. Columns represent mean (\pm SEM) number of actions elicited by each taste. Ingestive actions are paw licks (PL), lateral tongue protrusions (LTP), and rhythmic tongue protrusions (TP). Neutral or compromise responses are mouth movements (MM) or passive drip (PD). Aversive actions are gapes (G), chin rubs (CR), face washing (FW), forelimb flails (FF), headshakes (HS) and locomotion (LO). One, two, or three dots denote significance at 0.05, 0.02, and 0.01.

DISCUSSION

Does CDP promote eating by enhancing directly the positive palatability of foods? The present data suggest that it does. A shift in the consummatory action profile was seen for every taste stimulus after CDP administration. Ingestive actions were increased significantly each time. Aversive actions were less affected: aversion decreased significantly for only one taste, HCl, and sucrose showed an apparent but nonsignificant reduction. Quinine, the most reliable elicitor of aversive actions, showed no decrement in aversion after CDP although there was a clear increment in ingestive actions. The lack of a clear reduction in aversive responses is unlikely to be due to a floor effect, since at least some other pharmacologically-induced increases in positive palatability are accompanied by a complete elimination of aversive responses [4]. This implies that aversive actions in this experiment are remaining above their true floor.

The fact that positive actions are enhanced strongly by CDP while aversive actions are less reliably affected may indicate that CDP acts upon only half of the palatability evaluation mechanism. Recent reports have suggested that tastes activate two separate evaluation systems, which make independent decisions regarding, respectively, the positive and negative aspects of palatability [5,6]. These separate systems compete for expression in taste-elicited action. The hypothesis of separate palatability processing systems has received support from both neurological and pharmacological data [3,50]. The present data suggest that CDP acts to enhance the positive evaluation of palatability while having little or no effect on the aversive evaluation. The relatively small reduction in aversive responses to HCl (and possibly to sucrose) may be due to a competitive exclusion (or specific inhibition) of aversion by the enhanced positive evaluation (cf [6]). This interpretation is supported by the observation that quinine, which elicits a higher ratio of aversive to ingestive actions, shows no reduction in aversion after CDP even though ingestive actions are increased. In this case, the increased positive competition induced by CDP may not be sufficient to suppress a stronger constant level of aversion.

The suggestion that CDP enhances the positive palatability of foods carries important implications for interpretation of animal "models" of anti-anxiety drug action that involve motivation for food or water. For example, in the Geller conflict test, where responses are both reinforced with food and punished with electric shock, our data raise questions concerning whether the increase in responding observed after the administration of anti-anxiety agents is due to a specific inhibitory effect on fear motivation, a specific facilitatory effect on food motivation, or a complex mixture of both effects. This ambiguity of interpretation is most troublesome when the animal "model" is being used specifically to study the mechanisms of anti-anxiety drug action. Under these circumstances, it seems particularly important for the researcher to rule out the possibility that the drug effects observed in the animal test paradigm are due, in whole or in part, to an effect on processes more closely related to appetite rather than to anxiety (cf [16, 22, 40, 46, 47, 49]). In view of the present results, and the results of other studies, it might be more efficient to try to develop new animal measures of anti-anxiety effects that do not involve food motivation (e.g., [21, 25, 47], for a review see [46]).

Finally, the present results may have implications for current theories of the neural mechanisms of food motivation. It seems possible that some of the facilitatory effects upon positive palatability seen in the present investigation reflect an interaction of CDP with the putative "benzodiazepine-GABA receptor-ionophore complex" [33]. If the selective facilitatory effect of CDP upon positive consummatory reactions of rats can be attenuated with a specific benzodiazepine receptor blocker (e.g., RO 15-1788), the neuropharmacological specificity of the effect would have to be considered seriously. Clearly, such results would suggest an important new perspective from which to pursue the neurological mechanisms of feeding and drinking.

ACKNOWLEDGEMENTS

This work was supported by an NSERC operating grant (U0302) awarded to D. Treit, by NATO and Killam Foundation fellowships awarded to K. C. Berridge, and by NSERC (A9787) and MRC (MA7660) operating grants to J. C. Fentress, who generously provided laboratory facilities. We are indebted to J. C. Fentress and to M. L. Spetch for their helpful comments on the manuscript.

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