Pharmacology Biochemistry & Behavior, Vol 24, pp 677–685, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Pizotifen (BC-105) Attenuates Orienting and Pavlovian Heart Rate Conditioning in Rabbits[1]

SHERYL R GINN* AND D A POWELL[2]*†

**Neuroscience Laboratory, Wm Jennings Bryan Dorn Veterans' Hospital, Columbia, SC and Department of Psychology, University of South Carolina*
†Department of Psychiatry and Behavioral Science, University of South Carolina School of Medicine Columbia, SC 29208

Received 11 February 1985

GINN, S R AND D A POWELL *Pizotifen (BC-105) attenuates orienting and Pavlovian heart rate conditioning in rabbits* PHARMACOL BIOCHEM BEHAV 24(3) 677–685, 1986 —The cardiac component of the orienting reflex (OR) was elicited in rabbits by 75 dB, 4-sec duration tones of either 304 or 1216 Hz The conditioned cardiac response was also studied using the same tones and paraorbital electric shock as conditioned and unconditioned stimuli, respectively, using a differential Pavlovian conditioning paradigm Subcutaneous injections of the central 5-HT antagonist pizotifen (BC-105), the peripheral 5-HT antagonist xylamidine, the central 5-HT agonist d-lysergic acid diethylamide (LSD), and LSD in conjunction with BC-105 were administered 15 min prior to behavioral assessment Both the heart rate (HR) conditioned response (CR) and the OR consisted of bradycardia BC-105 attenuated, but xylamidine had no effect on, OR habituation LSD reduced the magnitude of the OR, an effect which was blocked by BC-105 BC-105 also produced a dose-related attenuation of the bradycardiac HR CR, however, xylamidine had no effect on HR conditioning, suggesting that the attenuation of the HR CR by BC-105 was central rather than peripheral in origin LSD potentiated the bradycardiac HR CR, but BC-105 in conjunction with LSD attenuated this response These results suggest that central 5-HT neurons may modulate the magnitude of bradycardiac responses during orienting and aversive Pavlovian conditioning

BC-105 d-LSD Xylamidine Orienting reflex Classical conditioning Heart rate Rabbits

ALTHOUGH skeletal Pavlovian conditioning requires many trials (100 to 200) before consistent conditioned responses (CRs) begin to occur, a number of non-specific responses, e g , cardiovascular adjustments, are acquired quite early (i e , within 10–20 trials) during training [11, 24, 39, 43, 48] The central nervous system (CNS) basis of these so-called "nonspecific CRs" may be important for understanding the neural substrates of learning and memory, since these CRs appear to be associated with the early events involved in information processing [6, 26, 35, 40, 42, 45]

Several lines of evidence suggest that forebrain structures are involved in cardiovascular conditioning (e g , [6,22]) These forebrain structures receive input from aminergic neurons via the medial forebrain bundle (MFB) [33], which may be involved in the mediation of cardiac CRs [10] Previous research has shown that cortical and/or hippocampal serotonin (5-HT) steady state levels were correlated with the magnitude of classically conditioned bradycardia in rabbits [17,18] Lateral hypothalamic lesions, which interrupted the MFB, were also found to produce depletion of forebrain 5-HT and norepinephrine (NE) concentrations in rabbits, as well as faster tonic baseline HR and smaller bradycardiac changes during adaptation, acquisition, and extinction of the Pavlovian conditioned eyeblink (EB) response [17] These results thus suggest that 5-HT and NE systems may be involved in the central mediation of learned cardiovascular responses as well as the bradycardia typically associated with the orienting reflex (OR)

Pitzotifen (BC-105) is a relatively specific 5-HT antagonist [28,29] Its mechanism of action is via 5-HT competitive receptor blockade [47], consequently central 5-HT mechanisms may be manipulated by its administration The purpose of the present experiment was thus to assess the effects of BC-105 on heart rate conditioning and the cardiac orienting reflex In order to better understand the mechanism of action of BC-105 on cardiovascular conditioned and unconditioned responses, a central 5-HT agonist was also tested It is known that the hallucinogen d-lysergic acid di-

[1]This research was supported by VA Institutional Research Funds awarded to the Wm Jennings Bryan Dorn Veterans' Hospital, Columbia, SC, and comprised a thesis submitted by the first author to the University of South Carolina in partial fulfillment of requirements for the M A degree

[2]Requests for reprints should be addressed to D A Powell, Ph D , Neuroscience Laboratory (151A), Wm Jennings Bryan Dorn VA Hospital, Columbia, SC 29201

ethylamide (LSD) is an agonist at both the S1 and S2 serotonin receptors [1] Separate experiments examined the effects of LSD alone and in conjunction with BC-105 on cardiac conditioning Other control experiments investigated the effects of the peripheral 5-HT antagonist xylamidine [8] on HR conditioning and the effects of BC-105 on cardiac and somatomotor unconditioned responses

METHOD

Subjects

Seventy-two experimentally naive albino rabbits (*Oryctolagus cuniculus*) of both sexes, approximately 6 months old and weighing approximately 2 5–3 75 kg were used The rabbits were housed in same sex pairs and maintained on free food and water throughout testing A 7 a m /7 p m light/dark cycle was maintained, with behavioral testing occurring during the light portion of this cycle

Apparatus

Behavioral testing was conducted for three consecutive days with four rabbits being tested simultaneously Each animal was tested in sound and light attenuating animal enclosures (Industrial Acoustics Co , Model AC-1), which were equipped with overhead houselights and speakers for delivery of auditory stimuli, and fans to provide ventilation and masking noise All experiments were controlled by a DEC minicomputer (PDP-11/10), which also collected and summarized the behavioral data directly from a Grass model 7D polygraph Heart rate was measured via Grass model 7P4D EKG-Tachograph preamplifiers set in the tachograph mode Electromyographic (EMG) activity was measured via Grass model 7P3 wide-band AC preamplifiers and integrators set in the integrator mode and calibrated such that a 1 mm pen deflection corresponded to a 100 μV potential across the recording pins The shock unconditioned stimulus (UCS) consisted of a 500 msec, 3 mA paraorbital AC shock train controlled by the computer For one-half of the subjects the CS+ was a 1216 Hz, 75 dB square-wave tone and the CS− was a 304 Hz, 75 dB square-wave tone The remaining subjects received the low tone as the CS+ and the high tone as CS− Tone duration was four seconds for all subjects The tones were produced by LM 555 multivibrators in conjunction with appropriate TTL circuits Tone onset and offset were controlled by the computer Shock onset coincided with tone offset for a 4-sec interstimulus interval

Response Measurement

EMG was measured via stainless steel No 4 insect pins inserted acutely into the neck muscles An EMG response was defined as a change of at least 200 μV from pre-CS baseline occurring during the 4-sec CS interval Trials on which EMG voltage was not at baseline at tone onset were not included in the data analysis HR was measured via stainless steel safety pins implanted subcutaneously on the right shoulder and left haunch Tachograph voltage from the polygraph amplifiers was sampled 16 times during each one-second interval These data were converted to instantaneous HR in beats per minute (bpm), which were then averaged within one second blocks Four 1-sec intervals were assessed prior to CS onset and eight 1-sec intervals were assessed after tone onset The four pre-CS measurements were averaged to yield a single pre-CS baseline (viz tonic) HR level The latter was then compared with each of the eight post-CS scores to determine the topography of the HR OR and CR as described below

Drugs

BC-105 (Sandoz) was suspended in sterile deionized water to inject as a suspension in a final volume of 1 ml/kg Xylamidine (Burroughs-Wellcome) and LSD (NIDA) were dissolved in deionized water to inject in a final volume of 1 ml/kg Control groups received physiological saline in a final injection volume of 1 ml/kg All drugs were injected subcutaneously (SC) 15 min prior to each experimental session Previous behavioral [49] as well as kinetic studies (e g , [47]) suggest that this time period should provide effective receptor blockade by BC-105 for the entire 3-hour conditioning session, as well as the initial OR testing period

Procedure

Each animal was allowed to become acclimated to the colony for at least three days The first day of testing consisted of orienting only Each animal was restrained in standard Plexiglas rabbit restrainers [14] and injected with the appropriate drug or vehicle Both HR and EMG were recorded No shocks were delivered Each animal was subjected to 15 trials consisting of a 4 0 sec tone with a 90 sec intertrial interval (ITI) One-half of the subjects in each group received a high tone (1216 Hz), the other half received a low tone (304 Hz) Determination of which animals received which tones was random with the stipulation that each sex be equally represented Following the orienting session the shock electrodes, which consisted of 11 mm stainless steel Michel wound clips, were attached approximately 0 5 cm above and below the rabbit's right eye The next two days were devoted to conditioning Acquisition sessions consisted of 128 trials with a 90 sec intertrial interval Tone duration was 4 0 sec The 304 Hz tones were presented on half of the trials, and the 1216 Hz tones were presented on the other half For half of the rabbits in each group, the low tone was paired with the shock UCS, as described above (CS+), while the high tone was never paired with the shock (CS−) These conditions were reversed for the other half The order of presentation of the tones was random with the stipulation that neither tone be presented more than three times in succession Animals were assigned randomly to one of nine groups as discussed below with the stipulation that an equal number of males and females be in each group

Experiment 1

This experiment examined the effects of four doses of BC-105 on HR conditioning and on the HR OR Thirty-two animals were used Each animal was assigned randomly to one of four groups (n=8) Group 1 received physiological saline (SC) (as above) and served as the control group Groups 2, 3, and 4 received 2 5, 5 0, or 10 0 mg/kg of BC-105 (SC), respectively All animals received differential HR conditioning and assessment of the cardiac component of the OR, as described above

Experiment 2

This experiment examined the effects of the peripheral antagonist xylamidine on HR conditioning and on the HR OR Sixteen rabbits were used One-half of the rabbits received the peripheral serotonin antagonist xylamidine (10 0

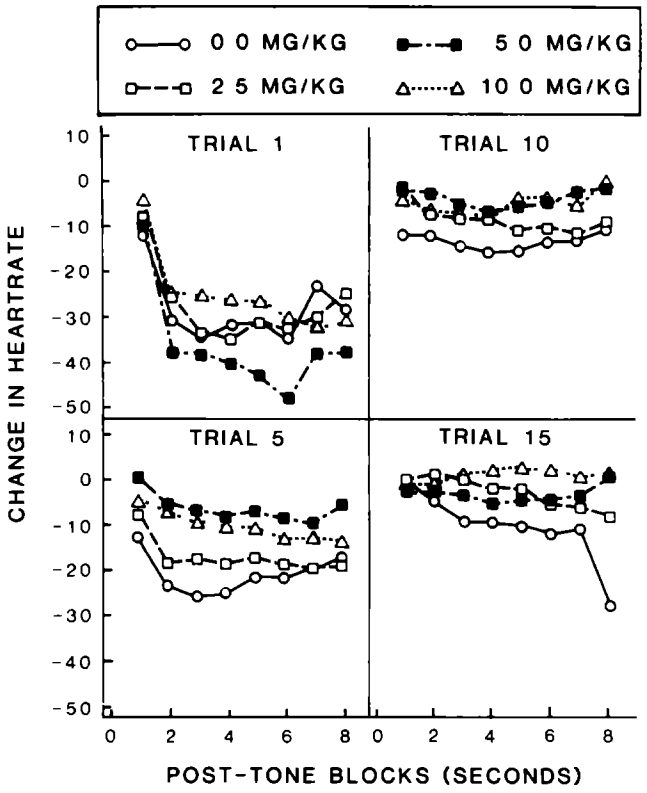

FIG 1 Change from baseline heart rate (beats per min) as a function of BC-105 dosage in response to 4 sec duration 75 dB unsignaled tones Data shown are for trials 1, 5, 10 and 15

mg/kg), while the remainder received physiological saline and served as a control group This dose of xylamidine was chosen based on both biochemical [8] and behavioral [49] evidence that it produces peripheral but not central 5-HT blockade In terms of relative potency it has been reported that it is comparable to the 5 mg/kg dose of BC-105 [8] All animals received differential HR conditioning and OR assessment, as described above

Experiment 3

This experiment examined the effects of the 5-HT agonist d-LSD on HR conditioning and the HR OR Twenty-four animals were assigned to one of three groups Group 1 received 0 03 mg/kg of LSD This dosage was based on a previous report [13], which indicated that it was within the range of maximal facilitation of nictitating membrane reflex (NMR) responding in rabbits Group 2 received 0 03 mg/kg LSD in conjunction with 5 0 mg/kg BC-105 The third group received physiological saline and served as a control group All animals received OR testing and HR conditioning as described above

Experiment 4

This experiment studied the effects of BC-105 on somatomotor and HR unconditioned responses to the shock UCS Fourteen animals, all of which had previously been exposed to the orienting and conditioning experiments, were studied Shock responsivity testing occurred at least one week after conditioning in order that residual drug effects would not affect performance One group (n=4) received physiological saline and served as the control group One group (n=4) received 2 5 mg/kg of BC-105 One group (n=3) received 5 0 mg/kg of BC-105, and the last group (n=3) received 10 mg/kg of BC-105 All drugs were administered subcutaneously 15 min prior to testing in a final volume of 1 ml/kg as in the orienting and conditioning experiments

Each animal received 60 presentations of unsignaled paraorbital electric shock at an intertrial interval of 15 sec Ten trials each of six different constant current shock intensities were presented in a random order Shock intensities were 0 0, 0 1, 0 2, 0 5, 1 0 and 2 0 mA, and shock duration was 500 msec Four somatomotor responses were scored by a trained observer through a one-way window in the experimental chamber as (a) no response, (b) a "twitch' (movement of the eyelids but failure to close them), (c) a ' blink" (a closure of the eyelids), or (d) a "jump" (a blink accompanied by movement of the entire body) "Threshold" shock intensities for twitch, blink and jump responses were calculated (in mA) as that intensity which elicited the response 50% of the time according to the method of constant stimuli [16] This procedure has been described in a previous report [41] Baseline HR was measured (UCR) for 1 sec preceding shock onset The HR unconditioned response was measured during four 1-sec blocks following shock termination These five HR scores were analyzed for the first, fifth and tenth presentations of the 0 2, 1 0 and 2 0 mA shocks during the session HR data were scored by hand from the polygraph record due to the random order of shock intensities and the difficulty of maintaining tachograph signals during unsignalled shock presentations

Data Analysis

The cardiac OR was analyzed by a mixed design analysis of variance (ANOVA) with drug group as a between-subjects measure and trials (15 levels) and 1-sec intervals (9 levels) as repeated measures The HR conditioning data were analyzed using a mixed design ANOVA with drug group again as a between-subjects measure, sessions (2 levels), CS (2 levels), test trial (3 levels), and blocks of 1-sec intervals (9 levels, viz 1 averaged pre-CS block and 8 post-CS blocks) as repeated measures HR scores were analyzed for test trials only, during which the UCS was omitted These test trials occurred on every eighth trial during the session, with the two tones alternating HR scores were analyzed for the first, fifth, and eighth test trial for each tone in order to assess HR change over time The HR was subsequently analyzed separately for each of the eight 1-sec post-CS intervals to determine the specific periods of time after CS onset during which significant effects of drug administration may have occurred The data analyzed consisted of change from baseline HR scores for each post-CS 1-sec block This procedure involved subtracting each post-CS mean from the pre-CS mean for each animal in each group Duncan's Multiple Range Test was used to post-test significant main effects and interactions

Since relatively few EMG CRs occurred, these data were analyzed using chi-square procedures Frequency of responding for each group was tabulated for both the orienting and acquisition sessions In order to analyze EMG amplitude, EMG frequency data were pooled into three

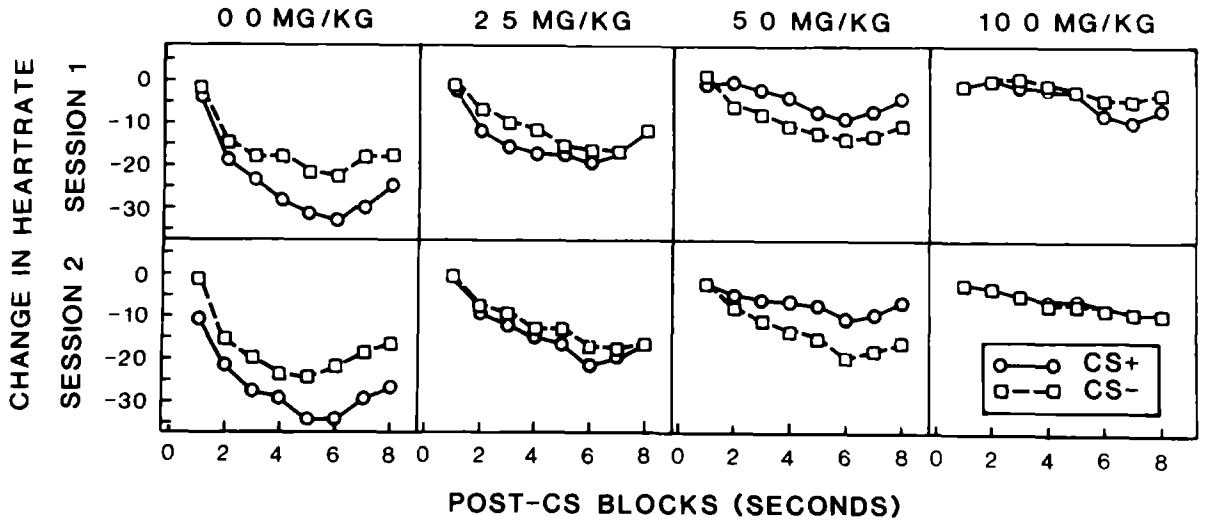

FIG 2 Change from pre-CS baseline heart rate (beats per min) of rabbits administered different doses of BC-105 in response to CS+ (tones paired with paraorbital shock) and CS− (tones not paired with shock) during two sessions of differential HR conditioning

categories (a) responses with amplitudes from 0 to less than 500 μV, (b) responses with amplitudes from 500 to less than 1000 μV, and (c) responses with amplitudes from 1000 μV to 5000 μV Chi square analyses were conducted on the number of subjects in each group that showed a response which fell into one of these three categories

RESULTS

Experiment 1 Effects of BC-105 on the Cardiac OR and on Pavlovian HR Conditioning

Orienting BC-105 had no effect on baseline HR, defined as the mean HR score during the pre-CS 4-sec interval, $F(3,28)=2\ 07$, $p=0\ 12$ Baseline HR scores (in bpm) were 233 8, 233 3, 205 7, and 204 3 (SEM=±11 5) for the saline, 2 5, 5 0 and 10 0 mg/kg BC-105 groups, respectively

The HR change from baseline for the four drug groups during orienting for trials 1, 5, 10 and 15 are shown in Fig 1 As has been previously reported [39], the cardiac component of the orienting reflex to novel tones consisted of bradycardia, which declined in magnitude as a function of subsequent presentation of the tones Figure 1 also suggests that BC-105 attenuated the magnitude of the response, but this effect did not appear to be dose-related Both the block (of 1-sec post-CS intervals), $F(8,224)=42\ 55$, $p<0\ 0001$, and trial, $F(14,324)=5\ 72$, $p<0\ 0001$, effects were significant, but the group effect was not, $F(3,28)=1\ 40$, $p>0\ 10$ The group by trial interaction approached, but did not quite reach the 0 05 level of significance, $F(24,224)=1\ 52$, $p<0\ 0628$ Nevertheless, separate ANOVAs of post-CS 1-sec intervals revealed that significant group effects occurred for many post-CS 1-sec blocks The saline control group was significantly different from the 5 0 and 10 0 mg/kg groups for blocks 1 and 2, and 5–9. Furthermore, the 2 5 mg/kg group was significantly different from the 5 0 and 10 0 mg/kg groups for blocks 1–9, and the 5 0 and 10 0 mg/kg groups were significantly different from each other for blocks 4–8 These findings thus suggest that the attenuation of OR magnitude by BC-105, as

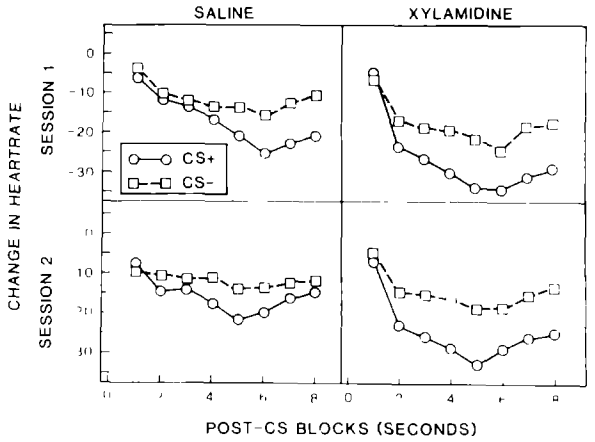

FIG 3 Change from pre-CS baseline heart rate (beats per min) of rabbits administered xylamidine (10 mg/kg) and saline (0 mg/kg) in response to CS+ (tones paired with paraorbital shock) and CS− (tones not paired with shock) during two sessions of differential heart rate Pavlovian conditioning

depicted in Fig 1, was reliable However, since the interaction of trials and group was not statistically significant at usually acceptable confidence levels, these findings must be interpreted with caution

Conditioning BC-105 had no effect on baseline HR, $F(3,28)=0\ 33$, $p=0\ 80$ Mean (SEM=±9 19 bpm) baseline HR scores (in bpm) were 231 2, 240 0, 231 8, and 227 6 for the saline, 2 5, 5 0 and 10 0 mg/kg BC-105 groups, respectively

Heart rate change from baseline for each group, CS and session is shown in Fig 2 The cardiac response consisted of HR slowing, which began during the first 1-sec interval following CS onset, and reached its maximum magnitude during post-CS interval 6 BC-105 attenuated the response to both the CS+ and CS− as well as the discrimination between

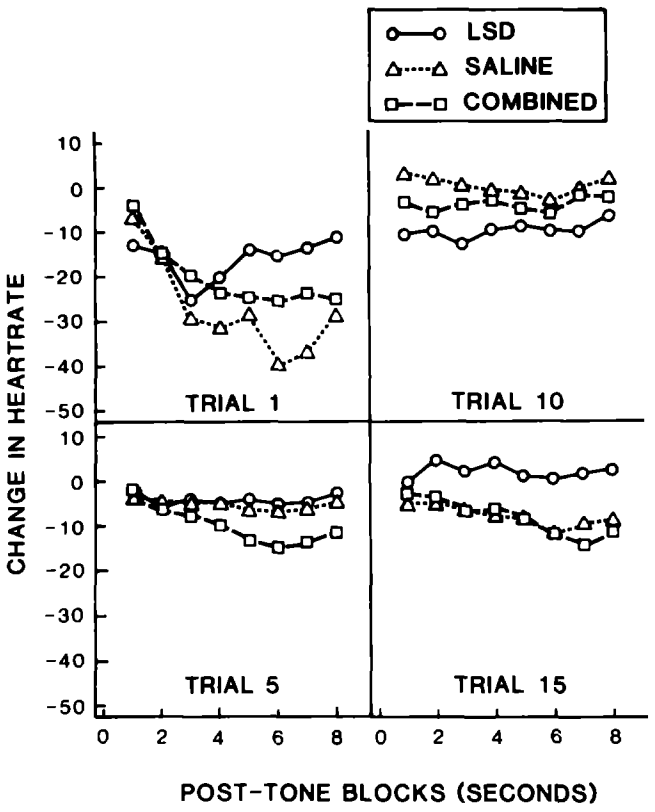

FIG 4 Change from baseline heart rate (beats per min) of rabbits administered LSD, LSD + BC-105 (combined) or saline in response to unsignaled tones of 4 sec duration and 75 dB intensity Data shown are for trials 1, 5, 10 and 15

the CS+ and CS− Moreover, this attenuation was dose related The smallest dose of BC-105 (2 5 mg/kg) had little effect on responding to the CS−, however, the response to the CS+ was attenuated at this low dose Only the largest dose (10 0 mg/kg) produced an effect on the response to the CS−

Both the CS, $F(1,28)=14\ 69$, $p<0\ 0001$, and blocks, $F(98,224)=60\ 95$, $p<0\ 0001$, effects were significant, suggesting that the bradycardia associated with the CS/UCS contingency was reliably greater to the CS+ than the CS− Moreover, two significant group interactions occurred group × blocks, $F(24,224)=5\ 50$, $p=0\ 0001$, and group × CS × blocks, $F(24,224)=2\ 22$, $p<0\ 001$ Analysis of each post-CS block indicated that there were significant group effects for post-CS intervals 2–8 (smallest, $F(3,28)=5\ 14$, $p<0\ 001$) Duncan post-tests showed that the saline control group was significantly different from (a) each of the three drug groups for post-CS intervals 2–6, and (b) both the 5 0 and 10 0 mg/kg groups for post-CS intervals 7 and 8 This analysis also indicated that the smallest dose of BC-105 (2 5 mg/kg) was significantly different from the largest dose (10 0 mg/kg) for post-CS intervals 2–7, although this dose was not different from the intermediate dose of BC-105 (5 0 mg/kg)

Separate ANOVAs also revealed that the group × CS interaction was significant for post-CS intervals 3–8 (smallest, $F(3,28)=2\ 96$, $p<0\ 05$) Duncan's Multiple Range Test revealed that the saline group's responses to the CS+ and CS− were significantly different from those of each of the other three groups over several of the post-CS intervals The 2 5 mg/kg group's responses to the CS+ and CS− were also significantly different from those of each of the other two drug groups during several post-CS intervals, with the exception of the 5 0 mg/kg group for the CS− condition Significant differences between the 5 0 mg/kg and 10 0 mg/kg groups were also obtained for CS+ and CS− on several post-CS intervals

EMG There were no significant differences between any of the four groups on EMG responding during orienting or session 1 of acquisition However, during session 2, a significant effect was observed for both CS+, $\chi^2(3)=16{,}001$, $p<0\ 005$, and CS−, $\chi^2(3)=9\ 0255$, $p<0\ 05$, due to greater responding in the 5 0 mg/kg group

Experiment 2 Effects of Xylamidine on the Cardiac OR and on Pavlovian HR Conditioning

Orienting Mean baseline HR was not affected by xylamidine during OR assessment, $F(1,14)=0\ 24$, $p=0\ 63$ Mean baseline HR scores were 225 1 and 218 7 (SEM = ±9 24) for the xylamidine and saline groups, respectively The HR change from pretone baseline (i e , OR) and its habituation over trials were also unaffected by xylamidine However, both block, $F(8,112)=14\ 67$, $p<0\ 0001$, and trial, $F(14,112)=2\ 3$, $p<0\ 01$, effects were significant, indicating that the OR and its habituation over trials was normal

Conditioning Xylamidine had no effect on baseline HR during conditioning, $F(1,14)=0\ 37$, $p=0\ 55$ Mean baseline HR scores were 217 2 and 210 1 (SEM = ±8 33) for xylamidine and saline, respectively

The change from baseline HR for the two groups for CS+ and CS− over sessions is shown in Fig 3 Xylamidine appeared to potentiate the deceleration to the CS+, however, it had little effect on the response to the CS− Although the group effect was not statistically significant, $F(1,14)=0\ 02$, $p=0\ 89$, a significant group × block effect occurred, $F(8,112)=2\ 00$, $p=0\ 05$ The two groups were significantly different from each other for blocks 1 and 2 Blocks and the CS+/CS− dimension were also significant, $F(8,112)=29\ 94$, $p<0\ 0001$, and $F(1,14)=14\ 03$, $p<0\ 002$, respectively, but no other significant group effects or group interactions were found

EMG No differences were found between the two groups for EMG responses during either orienting or acquisition

Experiment 3 Effects of BC-105 in Combination With d-LSD on the Cardiac OR and on Pavlovian HR Conditioning

Orienting There was a difference in baseline HR among the three groups, $F(2,21)=19\ 69$, $p<0\ 001$ The mean baseline HR scores were 269 8, 198 7, and 215 2 (SEM = ±8 32) for LSD, LSD and BC-105, and saline, respectively

HR change from baseline for trials 1, 5, 10 and 15 is presented in Fig 4 LSD appeared to facilitate habituation to the tone This effect had occurred by trial 5, and was maximal at trial 15 In fact, by trial 15, the response in the LSD group had become accelerative LSD and BC-105 likewise appeared to cause rapid habituation, however, there was little difference between this group and the saline group by trial 15

Again significant blocks, $F(8,168)=9\ 07$, $p<0\ 0001$, and

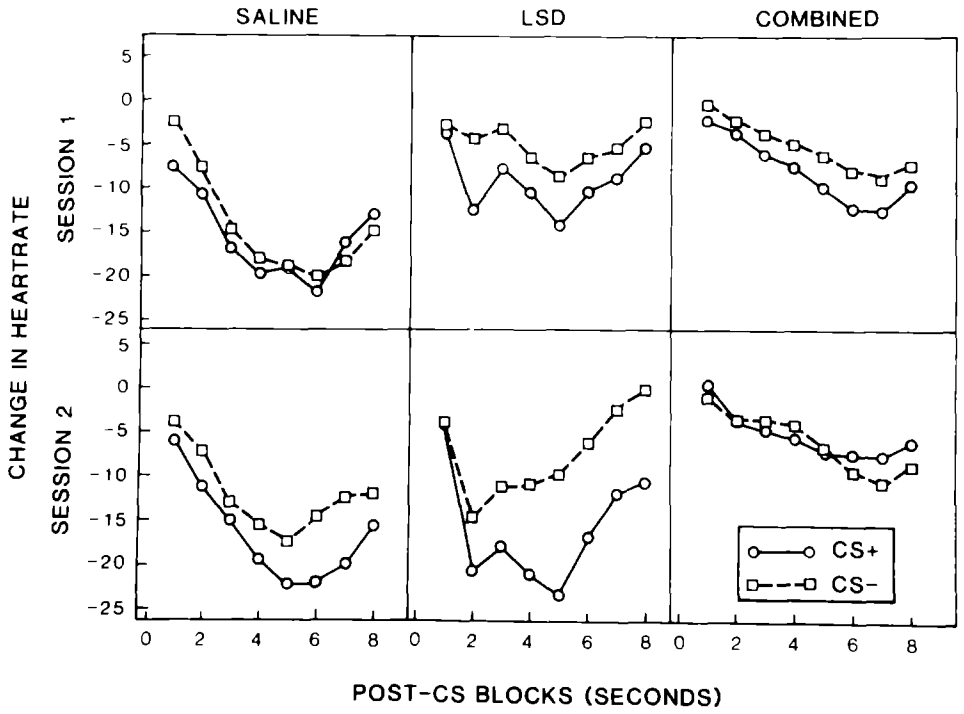

FIG 5 Change from pre-CS baseline heart rate (beats per min) of rabbits administered LSD LSD + BC-105 (combined) or saline in response to CS+ (tones paired with paraorbital shock) and CS− (tones not paired with shock) during two sessions of differential HR conditioning

TABLE 1

MEAN PARAORBITAL SHOCK 'THRESHOLDS' OF RABBITS TREATED WITH BC-105 (± SEM)

	Response		
Drug Group	Twitch	Blink	Jump
Saline	0 155 ± 0 04	0 843 ± 0 14	2 62 ± 0 31
2 5 mg/kg	0 092 ± 0 01	0 410 ± 0 12	2 45 ± 0 22
5 0 mg/kg	0 113 ± 0 01	0 987 ± 0 497	2 59 ± 0 41
10 0 mg/kg	0 1167 ± 0 03	0 740 ± 0 21	2 38 ± 0 30

trials, $F(14,288)=1\ 8$, $p<0\ 04$, effects occurred, suggesting that the change in HR associated with orienting as well as its habituation was reliable Significant effects for group × block, $F(16,168)=272$, $p<0\ 001$, group × trial, $F(28,288)=1\ 70$, $p<0\ 05$, and group × trial × block, $F(224,2293)=1\ 53$, $p<0.001$, were also found Duncan's Multiple Range Test indicated that the LSD group was significantly different from the combined group as well as the saline group for all eight blocks of HR during trial 1, and, for trials 5, 10, and 15, all three groups were significantly different from each other for all post tone blocks of HR

Conditioning There were no differences between the three groups on baseline HR during conditioning, $F(2,21)=0\ 23$, $p=0\ 798$ Mean baseline HR for the groups were 220 98, 214 08, and 218 79 (SEM=±7 38) for LSD, LSD and BC-105, and saline, respectively

Change from baseline HR for the three groups is presented in Fig 5 In general LSD appeared to potentiate responding to the CS+, compared to the CS− Differences between CS+ and CS− responding appear greater in the LSD group than in either the saline or combined groups However, compared to the saline group, the magnitude of the deceleration to CS+ and CS− in the LSD group was attenuated during session 1, but not session 2 Moreover, the response was somewhat slower to develop in the saline and combined groups compared to the LSD group The response of the latter group had almost reached its greatest magnitude by the second 1-sec post-CS interval, whereas response development was much more gradual in the saline and combined groups, which did not reach their largest magnitude change until post-CS block 6 or 7 BC-105 appeared to block the potentiation of the response by LSD, especially on the second day of acquisition As in Experiments 1 and 2, significant blocks, $F(8,168)=37\ 36$, $p<0\ 0001$, and CS, $F(1,21)=19\ 6$, $p<0\ 0002$, effects occurred, suggesting that the HR slowing associated with the CS/UCS contingency was reliably greater in response to the CS+ than the CS− A significant interaction for group × block also occurred, $F(16,168)=5\ 57$, $p=0\ 0001$ Separate ANOVAs computed for each block showed a significant group effect for post-CS intervals 2–8 (smallest, $F(2,21)=3\ 74$, $p<0\ 05$) Duncan's Multiple Range Test revealed that (a) the LSD group was significantly different from the saline group for post-CS intervals 6, 7 and 8, (b) the combined LSD and BC-105 group was significantly different from the saline group for post-CS intervals 3, 4, 5 and 6, and (c) LSD was significantly different

from the combined group for post-CS interval 2 A significant group × CS effect was observed only for post-CS interval 5, $F(2,21)=3\ 86, p<0\ 05$ Duncan post-tests revealed that the LSD group was significantly different from the combined group for CS+ only, and LSD was significantly different from the saline group for CS− only However, the combined group was significantly different from the saline group for both CS+ and CS−

EMG During orienting the LSD group showed more frequent and larger amplitude EMG responses than either of the other groups, $\chi^2(2)=13\ 67$, $p<0\ 005$, $\chi^2(4)=13\ 93$, $p=0\ 01$, respectively The combined BC-105 and LSD group never responded during orienting Only one saline animal responded No significant effects were found for any of the three groups during conditioning

Experiment 4 Effects of BC-105 on Unconditioned HR and Somatomotor Responses to Unsignalled Electric Shock

Somatomotor responses to the UCS are presented in Table 1 These data indicate the intensity of the UCS at which a response was elicited 50% of the time There were no significant differences among the groups for any of the three responses

There were also no significant differences in baseline (pre-shock) HR among the four groups Mean baseline HR scores were 202 9, 200 2, 200 1 and 197 9 (SEM = ± 17 02) for the 0 0, 2 5, 5 0 and 10 0 mg/kg groups, respectively

The HR UCR consisted of HR accelerations at all shock intensities for all 4 groups These accelerations had returned to baseline by the end of the post-UCS interval However, BC-105 dramatically increased the magnitude of this acceleration This was especially true of the 10 mg/kg group which revealed post-shock HR increases 3 to 4 times larger than the saline group at the highest shock intensity This finding resulted in a significant group × intensity × block effect, $F(24,80)=1\ 63$, $p=0\ 05$ Duncan's Multiple Range Test showed that (a) at the lowest shock intensity (0 2 mA) the saline and the 2 5 mg/kg groups were significantly different from the 10 0 mg/kg group for blocks 4 and 5, and the 2 5 mg/kg group was significantly different from the 5 0 mg/kg group for block 4, (b) at the 1 0 mA intensity the saline group was significantly different from the 10 0 mg/kg group but only during block 4, and (c) at the highest shock intensity (2 0 mA) the saline group was significantly different from all three drug groups during several post-shock blocks, and the 2 5 and 5 0 mg/kg groups were also significantly different from the 10 0 mg/kg for several post-shock blocks It should be noted that these data were not obtained on naive animals, however All had previously experienced shock in this situation which may have resulted in some pain-induced analgesia However, presumably this effect was similar for all groups of animals

DISCUSSION

The results of the present experiments suggest that BC-105 attenuates the bradycardia associated with CNS processing of information The cardiac component of the response to novel auditory stimuli (viz the OR) as well as the response to a CS/UCS Pavlovian conditioning contingency was attenuated by BC-105 administration BC-105 is most probably a specific antagonist for the 5-HT_2 receptor [29,30] These receptors are found predominantly in the limbic system, which has been consistently associated with higher cognitive function [6, 22, 32, 48] The finding that BC-105 produced a dose-related decrease in the magnitude of the cardiac CR thus may mean that 5-HT plays a role in the modulation of the cells within the limbic system during CNS processing of stimuli for information value However, it is well known that vascular smooth muscle also contains serotonin receptors which have been classified as 5-HT_2 [7, 29, 30, 37, 38] Thus the effects observed following the administration of BC-105 could have resulted from its influence on 5-HT receptors located in vascular smooth muscle However, the serotonin antagonist xylamidine, which has been found to produce primarily peripheral effects (i e , only minute quantities cross the blood brain barrier [8]), did not impair either the HR discrimination or HR CR magnitude In fact its effects were opposite to those produced by BC-105, suggesting that the attenuation of the heart rate CR by BC-105 was most probably central in origin

The results of Experiment 3 support the hypothesis that the effects of BC-105 on both the HR CR and OR may at least partially involve central 5-HT mechanisms The 5-HT agonist LSD produced an enhancement of the heart rate discrimination, which was opposite to the effects produced by the central antagonist BC-105 Thus, the data of Experiment 3 suggest that LSD facilitated the ability of the animal to discriminate between the two stimuli This finding is compatible with the finding by Gormezano and Harvey [15] that perhaps LSD affects the associative properties involved in the acquisition of somatomotor responses by influencing the CNS processing of the CS+ and CS− Unfortunately, the mechanism of action of LSD in the CNS is not well understood Serotonin is believed to mediate the effects of LSD [1,2], however, other neurotransmitter systems have not been ruled out [20] The results obtained in the present experiment could thus be explained by proposing that both LSD and BC-105 exert their effects at the receptors of these other neurotransmitters, e g , dopamine (also see below), although apparently LSD affects dopamine (DA) cells only at extremely large doses compared to its effects on 5-HT cells [20,21] Even more compelling, however, was the finding in Experiment 3 that BC-105 partially blocked the effects of LSD in the group which received both LSD and BC-105 This finding also suggests that the results obtained in Experiment 1 were mediated, at least in part, by central 5-HT mechanisms

The results of Experiment 4 support the conclusion that the effects of BC-105 on the HR CR cannot readily be interpreted as an effect on nociception The results of this experiment showed that BC-105 did not affect unconditioned somatic responding to the UCS and likewise had no effect on baseline HR, suggesting that the effects of BC-105 on HR conditioning observed in the previous experiments were not the result of an alteration in pain sensitivity Moreover, the HR UCR was an acceleration at all shock intensities, it was opposite in direction to the CR, a result which has been previously reported [23] In addition, BC-105 potentiated this response, unlike its effect on the CR, which consisted of both a decrease in discrimination and in CR magnitude This effect on the UCR may be mediated by an alteration in either an ascending or descending pain modulation system Serotonin is found in even greater concentrations in the spinal cord than in the brain [3] and much research suggests that these axons participate in a pain analgesia system [19,44] The application of 5-HT to dorsal horn cells depresses the response of these cells to noxious stimulation [5], thereby resulting in analgesia, an effect which is also induced by the

5-HT antagonist methysergide [50] It is thus possible that BC-105 blocked this 5-HT mediated analgesia

The finding that both LSD and BC-105 affected the magnitude of the OR and that LSD affected OR habituation, however, suggests that the effects produced by these drugs on conditioned responding may not be on an associative process per se Rather, it appears that unconditioned cardiac changes in response to novel stimuli, as well as stimuli which become signals through an associative process are affected by BC-105 as well as LSD Francis *et al* [10] reported that lateral hypothalamic (LH) lesions, which interrupted the MFB and resulted in 5-HT depletion, also attenuated the cardiac component of orienting as well as the cardiac CR This finding is compatible with the present results, assuming that the effects of BC-105 were due to 5-HT blockade, and that the effects of the LH lesions reported by Francis *et al* [10] resulted from the reported 5-HT depletion As described above, Hernandez *et al* [18,19] reported that cortical and hippocampal 5-HT resting levels were inversely correlated with the magnitude of the bradycardia occurring in response to Pavlovian conditioning contingencies, a finding that is also compatible with the present results

Leysen and co-workers [29] showed that BC-105 has a higher binding affinity for the 5-HT_2 than 5-HT_1 receptor However, these same researchers have demonstrated that BC-105 also has a high binding affinity for the H_1 (histamine 1) receptor Thus, the effects of BC-105 observed in Experiment 1 may have been due to a greater blockade of the H_1 rather than the 5-HT_2 receptor However, further research must be conducted in order to determine if histamine does, in fact, play a role in learned cardiovascular responses It is possible that histamine and serotonin exert synergistic effects at the same receptor An experiment in which 5-HT and histamine agonist and antagonist drugs are compared may ascertain the roles each of these monoamines play in learning

It can also be argued that the effects observed in these experiments may have been mediated by changes in sympathetic or parasympathetic preganglionic neurons 5-HT has been reported to produce excitatory effects at preganglionic sympathetic neurons [9] In contrast, reflex bradycardia in response to an elevation in arterial pressure is reduced by 5-HT [31] 5-HT is also reported to be a pressor agent in rats and rabbits because of its ability to increase sympathetic outflow (e g , [27]) However, 5-HT is reported to be a depressor agent in other species (e g , cats and dogs, [4]) The effects of serotonergic manipulations on blood pressure (BP) and HR are thus quite complex (for reviews, see [12,25]) However, in general, both HR and BP increase following the administration of 5-HT or 5-HT agonists In contrast, administration of 5-HT antagonists usually causes decreases in HR and BP However, the present results cannot be explained adequately in terms of decreased sympathetic activity due to BC-105 BC-105 did not decrease baseline HR, but produced a dose-related reduction in the magnitude of the HR CR The latter is a phasic bradycardiac response which is known to be due primarily to vagal influences [23] If the phasic effects observed were the result of a decrease in sympathetic activity, then changes should also have been observed in baseline HR Only LSD produced a significant change in baseline HR and this effect occurred only on the first day of testing, during OR assessment This effect probably occurred as a result of LSD's actions on the raphe system Stimulation of various raphe nuclei produces a variety of effects on BP and HR [34] It has been reported that electrical stimulation of the dorsal raphe nucleus (DRN) produces profound pressor effects [46] The DRN is also highly reactive to LSD Thus, the effects observed in Experiment 3 following LSD could have been the result of an increase in sympathetic outflow which resulted from increased activity in the raphe However, if this were the case, then subsequent doses of LSD should also have had comparable effects on baseline HR, yet LSD had no effects on baseline HR during conditioning Therefore, it appears most likely that changes in sympathetic activity did not mediate the effects of BC-105 and LSD on either the HR UCR or CR

In summary, it is clear that BC-105 attenuates the bradycardia associated with Pavlovian (classical) conditioning contingencies The control experiments with the peripheral 5-HT antagonist xylamidine and the 5-HT agonist LSD suggest that this attenuation may be due to its effects on CNS 5-HT systems, although future studies with other 5-HT antagonists as well as agonists will be necessary before this conclusion becomes unequivocally clear Similarly the present data suggest that BC-105 affects CNS processes controlling primary bradycardia, rather than a CNS associative process per se, since the unconditioned OR was also attenuated However, future studies with other 5-HT agents will also be required to completely clarify this issue

ACKNOWLEDGEMENTS

The authors thank Drs James Appel and Linda Hernandez for their assistance with this research The authors also thank Sandoz Pharmaceuticals, Inc , for the donation of the pizotifen (BC-105) and Burroughs-Wellcome Pharmaceuticals, Inc , for the donation of the xylamidine used in this study

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