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β-Adrenergic Manipulation in Amygdala Central N. Alters Rabbit Heart Rate Conditioning[1]

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GALLAGHER, M., B. S. KAPP, R. C. FRYSINGER AND P. R. RAPP. *β-Adrenergic manipulation in amygdala central n. alters rabbit heart rate conditioning.* PHARMAC. BIOCHEM. BEHAV. 12(3)419–426, 1980.—The present study was conducted to assess the effects of β-adrenergic manipulation within the central nucleus of the amygdala on Pavlovian heart rate conditioning in the rabbit. Administration of the β-adrenergic antagonist dl-propranolol into the central nucleus impaired the acquisition of conditioned heart rate responding compared to a vehicle injected control group. No significant effects of dl-propranolol on either baseline heart rate or on the heart rate orienting response were observed. The effect of dl-propranolol on conditioning exhibited stereospecificity, and animals receiving combined intracerebral injections of dl-propranolol and the β-adrenergic agonist l-isoproterenol did not exhibit comparable conditioning impairments. In addition, dl-propranolol administration dorsal to the central nucleus or into amygdala sites anterior or posterior to the central nucleus was less effective. These results support the interpretation that β-adrenergic activity within the central nucleus region of the amygdala complex contributes to the acquisition of classically conditioned heart rate responding.

β-Adrenergic receptors Amygdala central nucleus Heart rate conditioning Rabbits

INVESTIGATIONS aimed at understanding the physiological mechanisms which underly learning and memory indicate that a variety of brain structures participate in these processes. Determining the exact manner in which these different structures contribute to learning and memory will ultimately require a more precise description of the specific neural populations involved. For example, while it is well documented that the amygdala complex plays an important role in the acquisition and retention of aversive conditioning [14, 23, 29], this structure is composed of a number of different nuclei which possess distinct interconnections with other brain systems [19,20] and which exhibit considerable neurochemical heterogeneity [5, 6, 10, 16]. Consequently, research in our laboratory has focused on defining the contribution of specific amygdala systems to learning and memory processes based on nuclear grouping or neurochemical identity [12, 13, 18].

Our current research, using the intracranial administration of pharmacological agents, is aimed at investigating the intrinsic neural systems as well as the afferent and efferent connections through which the amygdala central nucleus influences the acquisition of Pavlovian conditioned heart rate responding in the rabbit. The rationale for the present experiment, which is designed to investigate a β-adrenergic system within the central nucleus, derives from several sources. First, our initial experiments have demonstrated that lesions confined to the rabbit central nucleus impair heart rate conditioning [18]. Second, results of research using pharmacological manipulations of noradrenergic activity have implicated brain noradrenergic systems in learning and memory [25, 26, 30]. Furthermore, using intracranial administration of noradrenergic agents we have previously demonstrated that β-adrenergic blockade within the amygdala impairs retention of aversive conditioning in rats [12]. The possibility that β-adrenergic manipulation within the amygdala impairs retention at least in part by altering norepinephrine activity within the central nucleus is suggested by recent neuroanatomical descriptions of the noradrenergic innervation of the amygdala complex [11, 17, 24, 31]. These studies using histochemical techniques indicate that noradrenergic input is unevenly distributed within the amygdala complex with the central nucleus receiving a particularly rich innervation. This experiment was therefore conducted to determine whether noradrenergic activity at β-adrenergic synapses within the region of the central nucleus contributes to the acquisition of conditioned heart rate responding in the rabbit.

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METHOD

Animals

Approximately 130 experimentally naive New Zealand albino rabbits (Canadian Breeding Farms and Laboratories, Ltd.) weighing from 2.2 to 2.7 Kg at the beginning of the experiment were used. All animals were maintained on a 12 hr light-dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) and were provided with food and water ad lib.

Surgery and Histology

All animals with the exception of those in unoperated control groups were pretreated with chlorpromazine hydrochloride (20 mg in 0.8 cc saline, IV) and anesthetized with Nembutal (30–75 mg, IV). They were mounted in a Kopf stereotaxic instrument fitted with a rabbit headholder, and bregma was adjusted 1.5 mm above the plane of lambda. Bilateral 23 g cannulae were implanted using the following coordinates: 0.1 mm anterior to bregma, 5.7 mm lateral to the midline and 11.8 mm ventral to dura. Animals in one group receiving cannula implants positioned dorsal to the amygdala were prepared using the above coordinates with the exception that cannulae were lowered 10.5 mm ventral to dura. Immediately following surgery all animals received intramuscular injections of Crysticillin (30,000 units, Squibb and Sons).

Following behavioral testing all animals were sacrificed and perfused with physiological saline followed by 10% formal-saline. Frozen sections (50 μ) were taken through the amygdala and stained with Thionin. Cannula tip placement was determined microscopically with the aid of the stereotaxic atlas of Urban and Richard [33]. Cannula tip placements for all groups except the dorsal placement group were rated as unacceptable if they were (1) more than 0.5 mm dorsal or ventral to the dorsal surface of the central nucleus; (2) anterior to the central nucleus as represented on plate A 18.5 mm of the Urban and Richard [33] atlas; (3) posterior to the central nucleus as represented on plate A 15.5 mm of the Urban and Richard [33] atlas. Cannula tip placements for the dorsal placement group were rated by the above criteria with the exception that a placement was rated unacceptable if it was less than 1.0 mm or more than 2.0 mm dorsal to the central nucleus. Only animals with bilaterally acceptable cannula placements were included in the data analysis.

Apparatus

The apparatus employed in this experiment was identical to that used previously [18]. During conditioning each animal was placed in a Plexiglas rabbit restrainer and position in one of four sound attenuating chambers within a shielded, soundproof, IAC room. Shock was delivered through stainless steel dresshooks attached to the upper and lower left eyelids. Stainless steel wire loops positioned subcutaneously, one dorsomedial to the left shoulder and one dorsomedial to the right haunch, were inserted shortly before the conditioning session to serve as EKG recording electrodes. The presentation of stimuli and recording of the EKG on an E and M Physiograph Six polygraph were controlled by solid state programming equipment.

Conditioning Procedure

Following 10–14 days of postoperative recovery, all animals were habituated to the Plexiglas restrainers for four daily one-half hour sessions followed on the fifth day by a one hour habituation session to the experimental chamber. Two days later the animals were placed into one of the four experimental chambers for the Pavlovian conditioning session. Fifteen presentations of the conditioned stimulus (CS), a 5.0 sec, 1000 Hz, 92 dB tone, were first presented using a random, variable, intertrial interval (80, 90, 100 sec; mean=90 sec). The presentation of fifteen CS alone trials prior to the onset of paired Pavlovian conditioning trials was used to habituate the decelerative heart rate orienting response. Without this habituation, any decelerative heart rate changes to the CS during the initial paired conditioning trials could represent, at least in part, orienting responses to a novel stimulus rather than true conditioned responses.

Immediately following the 15 CS alone trials 45 paired conditioning trials were presented, again using a random, variable, 90 sec intertrial interval. The offset of the CS was coincident with the onset of the unconditioned stimulus (US), a 500 msec, 2.0 mA eyelid shock.

Experimental Groups

The selection of the specific drug groups and the choice of drug doses used in this experiment were guided by our previous research [12]. Using varying drug concentrations injected into the amygdala of rats, the β-adrenergic antagonist dl-propranolol administered at approximately the concentration selected for this experiment (40 nmole) significantly impaired retention of passive avoidance conditioning. Furthermore, this effect in rats appeared to be due to the β-antagonist property of dl-propranolol because an equivalent dose of the dextro isomer of propranolol which has relatively weak β-blocking activity [4] did not significantly alter retention of avoidance conditioning. In addition, combined administration of dl-propranolol and l-norepinephrine significantly decreased the retention deficit produced by dl-propranolol administration. Based on these previous results it was predicted that in this experiment dl-propranolol administration into the central nucleus would alter conditioned heart rate responding in rabbits. In addition, if dl-propranolol altered heart rate conditioning by interfering with β-adrenergic activity it was predicted that d-propranolol would have significantly less effect and that the effects produced by dl-propranolol would be significantly attenuated by combined administration of dl-propranolol and the β-adrenergic agonist l-isoproterenol. Finally, if an effect on heart-rate conditioning was produced by interfering with β-adrenergic activity in the region of the central nucleus it was predicted that an equivalent dose of dl-propranolol injected dorsal to the central nucleus would be less effective. In order to test these predictions about the effects of dl-propranolol on heart rate conditioning in this experiment animals were assigned to one of six conditioning groups. Two control groups, an unoperated control group (UNOP PAIRED) and a vehicle injected group (VEHICLE), were included. Four drug injected groups included a group receiving dl-propranolol (40 nmole) into the central nucleus (DL-PAIRED), a group receiving d-propranolol (40 nmole) into the central nucleus (D-P), a group receiving combined injections of dl-propranolol (40 nmole) and l-isoproterenol (20 nmole) into the central nucleus, and a group receiving dl-propranolol (40 nmole) injections 1.0–2.0 mm dorsal to the central nucleus (DL-P DORSAL). The vehicle used for all injections was a Krebs-Ringer phosphate solution [32]. All injections were delivered bilat-

erally in a 1.0 μl volume approximately 2 min prior to the onset of behavioral testing.

Pseudoconditioning Procedure

To determine the extent of pseudoconditioning under the conditions used in this experiment and to assess the effects of dl-propranolol on the unconditioned response to the shock US, two additional groups were included. One group was an unoperated control group (UNOP, UNPAIRED) and the other group received dl-propranolol injections (40 nmole) bilaterally into the central nucleus (DL-P, UNPAIRED). Both groups were given 15 CS alone trials followed immediately by 45 presentations each of the CS and US in an unpaired, random manner. A random, variable, intertrial interval (35, 45, 55 sec, mean=45 sec) was used and no more than three presentations of either the CS or US occurred in succession.

Data Analysis

In order to provide a measure of the heart rate response to the tone CS, heart rate was recorded for the 5 sec preceding the presentation of the CS and for the duration of the 5 sec CS. The magnitude of heart rate change to the CS was computed by comparing the number of beats occurring during the 5 sec CS period with the number of beats occurring during the 5 sec pre-CS baseline period. The difference was expressed as percent change from the pre-CS baseline period.

To determine the unconditioned heart rate response to the US in the pseudoconditioning groups the duration for each of five consecutive blocks of five heart beats was measured immediately upon the offset of the US and expressed as a percent change from the mean duration of three successive blocks of five hearts occurring immediately before the onset of the US. Computing the percent change in heart rate for each of five successive five-beat intervals in this manner permits an analysis of the topography of the unconditioned response to the US.

A two factor (Groups×Trials) mixed design analysis of variance was used to analyze heart rate responses during conditioning. Pairwise, a priori comparisons were performed between groups according to the predictions which dictated the selection of groups included in this experiment. In addition, based on our previous research which demonstrated that the stimulus parameters used in this experiment produce maximal conditioned responses within 15 paired presentations of the CS and US [18], separate analyses of variance were performed on each 15 trial block in this experiment. The results of these 15 trial analyses provide information about the effects of pharmacological agents used in this experiment on both acquisition of conditioned responses and performance of conditioned responses at asymptote.

RESULTS

Histology

Histological inspection of cannula placements yielded bilaterally acceptable tip placements for eight animals in each group which were included in the data analysis. Cannula tip placements for an animal included in the DL-P PAIRED group are illustrated in Fig. 1. Twelve animals injected with dl-propranolol which did not have acceptable placements for inclusion in the DL-P PAIRED group nonetheless had bilaterally symmetrical cannula placements within the amygdala complex either anterior or posterior to the central nucleus. Since the data for these animals could provide additional information concerning the neuroanatomical specificity of the effects obtained with dl-propranolol administration into the central nucleus, these animals were assigned to one of three groups. One group (n=4) included animals with cannulae bilaterally positioned at the level of the anterior amygdala area anterior to the emergence of the medial and cortical nuclei. Another group (n=4) included animals with placements at the level of the medial and cortical nuclei but anterior to the emergence of the central and lateral nuclei. A final group (n=4) included placements posterior to the central nucleus at the level of the posterior lateral nucleus.

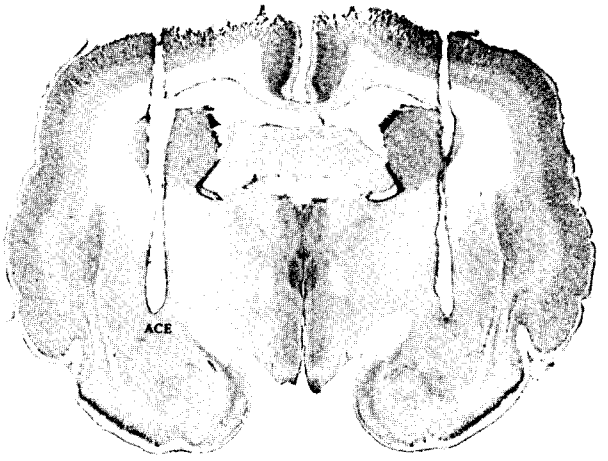

FIG. 1. Bilateral cannula placements for an animal included in the DL-P PAIRED group. ACE—Amygdala central nucleus.

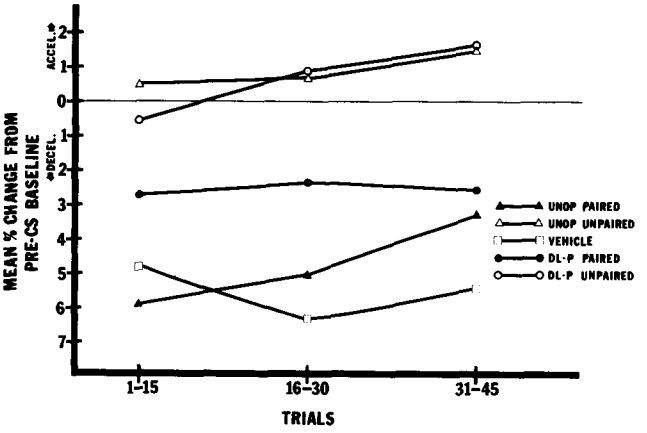

FIG. 2. Mean percent change to the CS from pre-CS baseline for groups UNOP PAIRED, VEHICLE, DL-P PAIRED, UNOP UNPAIRED, and DL-P UNPAIRED. Data points represent means for 15 trial blocks.

Conditioned Heart Rate Response

The heart rate responses to the CS for unoperated animals (group UNOP PAIRED) which received paired presentations of the CS and US and for unoperated animals (group UNOP PAIRED) which received unpaired presentations of these stimuli are presented in Fig. 2. A two factor (Groups× Trials) mixed design analysis of variance comparing the

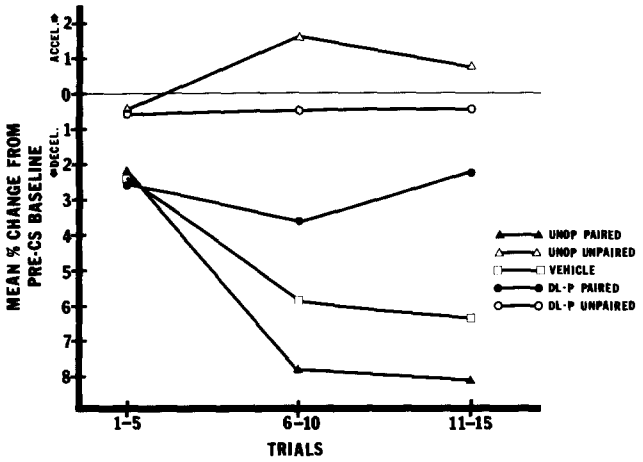

FIG. 3. Mean percent change to the CS from pre-CS baseline during the first 15 trials for groups UNOP PAIRED, VEHICLE, DL-P PAIRED, UNOP UNPAIRED and DL-P UNPAIRED. Data points represent means for 5 trial blocks.

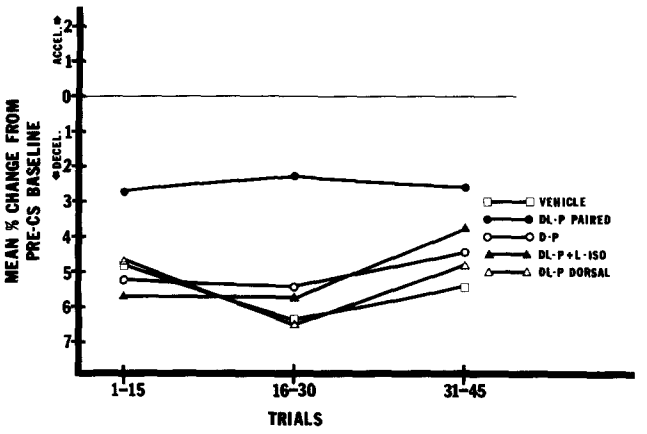

FIG. 4. Mean percent change to the CS from pre-CS baseline for groups VEHICLE, DL-P PAIRED, D-P, DL-P+L-ISO, and DL-P DORSAL. Data points represent means for 15 trial blocks.

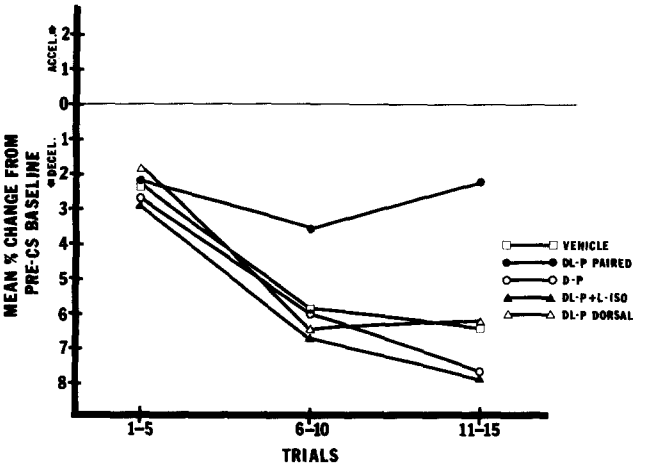

FIG. 5. Mean percent change to the CS from pre-CS baseline during the first 15 trials for groups VEHICLE, DL-P PAIRED, D-P, DL-P+L-ISO and DL-P DORSAL. Data points represent means for 5 trial blocks.

UNOP PAIRED and UNOP UNPAIRED groups was performed to determine the extent of pseudoconditioning in this experiment. This analysis revealed both a significant Groups effect, $F(1,14)=14.68$, $p<0.002$) and a significant Groups× Trials interaction, $F(44,607)=2.74$, $p<0.001$, indicating that the decelerative heart rate responses to the CS in the UNOP PAIRED group are clearly distinguishable from the responses of the UNOP UNPAIRED group. In addition, separate analysis of variance performed on these two groups for each of the 15 trials blocks represented in Fig. 2 indicated that acquisition of the conditioned heart response occurs rapidly during paired CS-US presentations. While significant Groups effects were obtained for each 15 trial block analysis, a significant Groups×Trials interaction was obtained only for the first block of 15 trials, $F(14,195)=7.14$, $p<0.001$. The acquisition of the conditioned response during the first 15 trials for the UNOP PAIRED group is illustrated in Fig. 3.

In order to determine if the surgical and vehicle injection procedures used in this experiment alter heart rate conditioning, analyses of variance comparing the vehicle injected control group (VEHICLE) and the unoperated control group (UNOP PAIRED) were performed for each 15 trial block. No significant differences emerged for Groups or for Groups× Trials interactions. The parallel development of conditioned responses for both the VEHICLE and UNOP PAIRED groups during the first 15 trials is illustrated in Fig. 3 and was reflected in a significant Trials effect during this initial block of trials, $F(14,196)=8.30$, $p<0.001$. These results indicate that the surgical and vehicle injection procedures used in this experiment do not alter significantly the acquisition of conditioned heart rate responding.

In order to determine the effects of dl-propranolol on heart rate conditioning groups, DL-P PAIRED and VEHICLE were compared. As illustrated in Fig. 2, animals (Group DL-P PAIRED) receiving propranolol injections into the central nucleus demonstrated an impairment in the conditioned decelerative heart rate response relative to the vehicle injected group (VEHICLE). Statistical analysis comparing these two groups revealed a significant Groups× Trials interaction for the first 15 trial block, $F(14,190)=1.95$, $p<0.02$, while Groups effects were significant for both the second and third blocks of 15 trials, $F(1,14)=8.51$, $p<0.01$; $F(1,14)=5.90$, $p<0.02$. The impaired acquisition of conditioned responses exhibited by the DL-P PAIRED group during the first 15 trials is illustrated in Fig. 3.

In order to determine whether the effects of dl-propranolol on heart rate conditioning exhibit stereospecificity, analyses were performed between the DL-P PAIRED group and the group receiving injections of the dextro-isomer of propranolol into the central nucleus (group D-P). As illustrated in Figs. 4 and 5, while dl-propranolol attenuated the decelerative response to the CS during conditioning, animals injected with an equivalent dose of d-propranolol exhibited decelerative responses to the CS during conditioning which closely parallel those of vehicle injected animals. This difference in the effects of dl-propranolol and d-propranolol was reflected in the results of analyses of variance comparing these groups which revealed significant effects for both Groups, $F(1,14)=6.51$, $p<0.02$, and the Groups×Trials interaction, $F(14,189)=2.31$, $p<0.01$, during the first 15 trials. In addition, no significant differ-

ences were obtained for analyses comparing the D-P group and the VEHICLE group for any of the 15 trials blocks. These results demonstrate that the effects produced by dl-propranolol administration are not produced by an equivalent dose of the dextro isomer which has relatively weak β-antagonist properties.

Additional support for the interpretation that dl-propranolol alters heart rate conditioning by interfering with β-adrenergic activity is provided by the data obtained for the group receiving combined injections of the β-antagonist dl-propranolol and the β-agonist l-isoproterenol (DL-P+L-ISO). Although analyses comparing the DL-P PAIRED and DL-P+L-ISO groups yielded group effects which only approached significance for the first two blocks of 15 trials, $F(1,14)=3.90 p<0.066$; $F(1,14)=3.85, p<0.07$, and yielded no significant effects for Groups × Trials interactions, comparison of the DL-P + L-ISO and VEHICLE groups yielded no significant Groups effects or Group×Trials interactions for any of the 15 trial block analyses. These results indicate that β-agonist administration partially attenuates the effects of dl-propranolol administration.

Comparisons between the DL-P PAIRED and DL-P DORSAL groups were performed to determine if the effects of dl-propranolol were produced by diffusion of the drug from the injection site to structures dorsal to the amygdala complex. Analyses of variance comparing the DL-P PAIRED and DL-P DORSAL groups yielded a significant Groups×Trials interaction during the first block of 15 trials and Groups effects were significant during the second and third blocks of 15 trials, $F(1,14)=7.74$, $p<0.02$; $F(1,14)=5.47$, $p<0.05$. In addition, analyses of variance comparing the DL-P DORSAL and VEHICLE groups did not reveal any significant differences between these groups for any of the 15 trial blocks. These results suggest that dl-propranolol does not alter heart rate conditioning by exerting its effects on regions dorsal to the central nucleus injection site.

Nonetheless, the possibility exists that dl-propranolol injected into the central nucleus may exert some or all of its effects on amygdala nuclei adjacent to the central nucleus. The data presented in Fig. 6 provide limited support for the notion that the central nucleus region is the primary site of action for dl-propranolol in this study. The data points which appear in Fig. 6 represent the mean conditioned response to the CS during the first 15 trials for individual animals injected with dl-propranolol (40 nmole). These data are plotted according to histologically confirmed cannula placements representing the anterior to posterior extent of the amygdala complex. Histological criteria for Groups 1, 2, and 5 were previously described in the histology results section. Groups 1 and 2 include animals with cannulae positioned anterior to the central nucleus at the levels represented in the atlas illustrations in Fig. 6. Group 5 includes animals with cannula placements posterior to the central nucleus at the level of the lateral posterior nucleus as represented in the atlas illustration. Groups 3 and 4 include animals whose bilateral placements met the criteria for inclusion in the DL-P PAIRED group. Group 3 includes animals with bilateral placements at the level of the central nucleus anterior to the emergence of the basolateral complex and Group 4 includes animals with central nucleus placements at the level of the basolateral complex. It is apparent that the conditioned responses for animals with cannula placements at the level of the central nucleus (Groups 3 and 4) are generally of smaller magnitude than those of animals with cannulae positioned within the amygdala either anterior or posterior to the central nucleus.

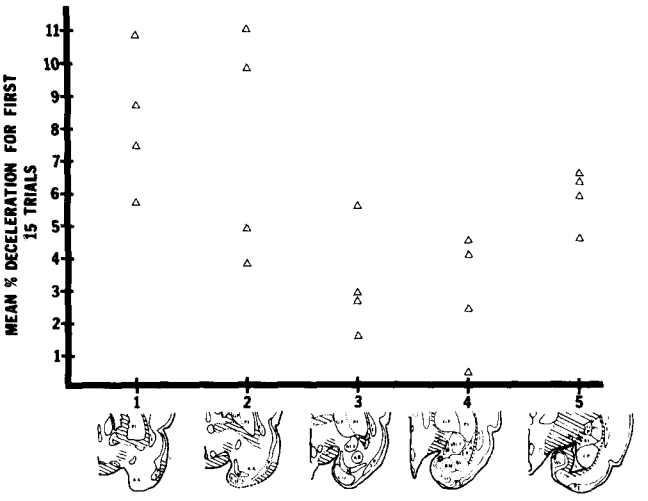

FIG. 6. Conditioned responses during the first 15 trials for animals injected with dl-propranolol. Each data point represents the mean decelerative heart rate response to the CS over 15 trials for an individual animal. Data points are plotted along the abscissa according to histologically confirmed cannula placements. The atlas illustrations for the five groups of cannula placements were adapted from the Urban and Richard [33] atlas and represent the anterior (Group 1) to posterior (Group 5) extent of the amygdala complex. Abbreviations: AA—anterior amygdala area, AB—amygdala basal n., AC—n. accumbens, ACE—amygdala central n., CL—claustrum, CO—amygdala cortical n., BL—amygdala basalateral n., BM—amygdala basalmedial n., GL—globus pallidus, HV—ventral hippocampus, LA—amygdala lateral n., LP—amygdala lateral posterior n., M—amygdala medial n., PU—putamen, PY—pyriform cortex, V—ventricle, ot—optic tract, st—stria terminalis.

Baseline Heart Rate

A measure of baseline heart rate was provided by the number of beats occurring during the 5 sec pre-CS period over the 45 trials. Baseline heart rate was compared for the UNOP PAIRED, VEHICLE and DL-P PAIRED groups. An analysis of variance yielded no significant Groups or Groups × Trials effects. The mean heart rate for each group was: UNOP PAIRED 210 beats/min, VEHICLE 209 beats/min, and DL-P PAIRED 209 beats/min. A significant Trials effect, $F(44,902)=3.21, p<0.001$, obtained in this analysis appeared to be due to a gradual decline in baseline heart rate for all groups over the early conditioning trials. Mean heart rates for all groups for each 5 trial block during the first 15 trials were 220, 210, 206 beats/min. Baseline heart rate following this initial decline remained relatively stable over the second and third blocks of 15 trials at 207 and 208 beats/min, respectively.

Heart Rate Orienting Response

An analysis of variance performed on percent change from baseline during the 15 CS alone presentations for the UNOP PAIRED, VEHICLE and DL-P PAIRED groups yielded no significant effects for either Groups or the Groups × Trials interaction. All groups exhibited a bradycardia response to initial presentations of the CS which habituated over trials as reflected in a significant Trials effects, $F(14,287)=12.17, p<0.001$.

Unconditioned Heart Rate Response

The unconditioned response to the US for the UNOP UNPAIRED group during the pseudoconditioning procedure consisted of a heart rate acceleration. Over the 45 US presentations this cardioacceleration for the unoperated animals reached peak magnitude consistently during the third five beat interval. The magnitude of cardioacceleration exhibited by unoperated animals decreased slightly over trials. The mean percent increases exhibited by ths UNOP UNPAIRED group during the third five beat interval for each of the 15 trial blocks were 16.9%, 15.1%, and 13.2%. The DL-P UNPAIRED group responded to the US with a cardioacceleration which closely resembled both in magnitude and topography the response of the UNOP UNPAIRED group during the initial US presentations. The mean percent heart rate increases during the third five beat interval over the first five US presentations for the UNOP UNPAIRED and DL-P UNPAIRED groups were 17.4% and 17.5%, respectively. However, analysis of variance comparing these two groups over 45 US presentations revealed significant effects for both Groups, $F(1,12)=7.92$, $p<0.02$, and the Groups×Trials, $F(8,96)=2.82$, $p<0.01$, interaction. These effects are attributable to the changing magnitude of the unconditioned response over trials primarily in the DL-P UNPAIRED group. While the peak heart rate increase in the DL-P UNPAIRED group appeared consistently in the third five beat interval, propranolol injected animals exhibited progressively less cardioacceleration over trials. The mean percent cardioacceleration exhibited by the DL-P UNPAIRED group during the third five beat interval for each 15 trial block was 11.6%, 4.8%, and 4.3%

DISCUSSION

Animals in the unoperated control groups in this experiment exhibited unconditioned and conditioned heart rate responses similar to those previously reported [18]. The heart rate orienting response to initial presentations of the tone CS alone consisted of a bradycardia which habituated rapidly with repeated CS presentations. During paired presentations of the CS and US conditioned decelerative heart rate responses developed rapidly in the UNOP PAIRED group whereas the UNOP UNPAIRED group did not exhibit comparable responses to the CS during unpaired stimulus presentations. These results confirm our previous findings indicating that responding in the UNOP PAIRED group is not attributable to pseudoconditioning. Finally, the unoperated animals exhibited an unconditioned response to the shock US which consisted of heart rate acceleration. We have previously reported that at the stimulus parameters used in this experiment, rabbits exhibit a cardioacceleration to shock which decreases in magnitude over the course of the 45 US presentations. The magnitude of the cardioacceleration exhibited by unoperated animals during early presentations of the US in this experiment closely approximates that previously observed. However, compared to our previous report, the UNOP UNPAIRED group exhibited relatively less habituation of the heart rate acceleration response. At present we cannot account for this disparity in the degree of habituation observed.

The results of the present experiment indicate that dl-propranolol administration into the central nucleus does not significantly alter either baseline heart rate or the heart rate orienting response to tone CS presentations. However, dl-propranolol administration into the central nucleus appears to alter heart rate responding to the US. Compared to the UNOP UNPAIRED group, animals in the DL-P UNPAIRED group exhibited progressively less cardioacceleration to the shock over trials. Although the significance of this difference in the response of unoperated and dl-propranolol injected animal to shock remains an interesting question for future research, in the context of the present experiment it should be emphasized that both groups exhibited a similar topography and magnitude of unconditioned responding during the early presentations of the US. These results indicate that during the initial US presentations both groups react similarly and that the impaired acquisition of conditioned heart rate in the DL-P PAIRED group is probably not attributable to gross changes in sensitivity to the noxious US.

The results of this experiment also demonstrate that dl-propranolol administration into the central nucleus of the amygdala complex impairs the acquisition of classically conditioned heart rate responding. During the first 15 paired CS-US presentations the DL-P PAIRED group exhibited a significant reduction in the magnitude of conditioned responses compared to the vehicle injected group. Furthermore, dl-propranolol did not simply delay the acquisition of conditioned responses since responses to the CS in the DL-P PAIRED group did not exhibit further increases during subsequent trials. This effect on conditioning appears to be due to the β-adrenergic antagonist property of dl-propranolol because an equivalent dose of d-propranolol, which possesses only weak β-antagonist activity, does not significantly alter conditioning. Furthermore, the acquisition of conditioned responding exhibited by animals receiving combined administration of dl-propranolol and the β-adrenergic agonist l-isoproterenol did not significantly differ from the vehicle control group. These pharmacological effects are consistent with other research demonstrating that β-adrenergic receptors identified in the central nervous system exhibit pharmacological properties similar to peripherally located β-adrenergic receptors [1,7]. In addition, β-adrenergic receptors have been localized within the amygdala complex [7,22]. Therefore, the results obtained in this experiment support the interpretation that activity at β-adrenergic synapses in the amygdala contributes to the acquisition of heart rate conditioned responding in the rabbit.

In addition, the evidence provided in this experiment supports the interpretation that dl-propranolol injected into the central nucleus produced its effects on conditioning by altering β-adrenergic activity within the target region. Injections of dl-propranolol dorsal to the central nucleus were ineffective and, compared to administration into the central nucleus, injections at cannula sites anterior or posterior to the central nucleus produced less or no apparent effect on conditioned responding. However, the possibility remains that the effects of dl-propranolol on heart rate conditioning are not exclusively mediated within the central nucleus proper. Based on research using histochemical techniques the dorsal rostral regions within the amygdala complex which receive the most prominent norepinephrine input include the central nucleus and the closely adjacent dorsal portion of the basolateral nucleus [31]. It is, therefore, possible that dl-propranolol produces its effects at these norepinephrine terminals adjacent to the central nucleus. However, in this context it is interesting to note the close similarities between the effects on conditioned and unconditioned heart rate responses produced by dl-propranolol administration into the central nucleus in this experiment and by lesions confined to the central nucleus in our previous

research [18]. Both experimental manipulations impair the acquisition of classically conditioned responses without significantly altering either baseline heart rate or the unconditioned heart rate orienting response.

Whether noradrenergic activity within the central nucleus region contributes to the acquisition of classically conditioned heart rate responding by contributing to sensory, motivational or associative processes is not unequivocally demonstrated in this experiment. Since the orienting response to the CS and the initial unconditioned response to the shock US is unaffected by dl-propranolol, the effects of dl-propranolol on conditioning are probably not attributable to gross alterations in sensory processing mechanisms. However, the interpretation that β-adrenergic blockade within the amygdala produces its effects on conditioning by altering associative and/or motivational processes receives some support from our previous observations that the same pharmacological manipulations performed in this experiment alter retention of aversive conditioning in rats when drugs are administered into the amygdala after the training experience [12]. The exact role of β-adrenergic activity within the central nucleus in motivational and/or associative processes will require further research.

Finally, these data suggest that activity at β-adrenergic synapses within the central nucleus region plays a role in the acquisition of conditioned heart rate responding. The identification of such a neuroanatomically and neurochemically specified population of neurons may aid ultimately in providing a more detailed and complete description of the neural circuitry involved in conditioning processes. For example, based on the evidence provided in this experiment an important issue regards the origin of the norepinephrine input which contributes to the acquisition of heart rate conditioned responding. Recent research has demonstrated that the central nucleus region of the amygdala receives dual innervation from the locus coeruleus and from noradrenergic cells of medullary origin [11]. While a possible role for the locus coeruleus noradrenergic system in learning and memory processes has been the focus of considerable attention and debate [2, 3, 8, 21, 28], relatively little research has been directed at understanding the function of other noradrenergic projections to the forebrain [15]. The possible contribution of medullary catecholamine systems to conditioning of cardiovascular responses is particularly interesting in light of the possibility that at least a component of the medulllary catecholamine projection to the central nucleus may derive from cells in the A2 region. Through other lines of investigation, this specific collection of adrenergic cells in association with the nucleus of the solitary tract has been implicated in cardiovascular regulation [9, 27, 34]. Therefore, an interesting focus for future research would be to determine the relative contribution of different norepinephrine inputs into the amygdala to heart rate conditioning.

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