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Selective Autonomic Blockade of Conditioned and Unconditioned Heart Rate Changes in Rabbits[1]

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FREDERICKS, A., J. W. MOORE, F. U. METCALF, J. S. SCHWABER AND N. SCHNEIDERMAN. *Selective autonomic blockade of conditioned and unconditioned heart rate changes in rabbits.* PHARMAC. BIOCHEM. BEHAV. 2(4) 493–501, 1974. – Autonomic blockades were used to examine relationships between heart rate conditioned responses (CRs) and unconditioned responses (URs) during differential classical conditioning in rabbits. Electrical stimulation of septal region or hypothalamus through chronically implanted electrodes was the unconditioned stimulus (US); conditioned stimuli (CSs) were intracranial stimulation of left and right lateral geniculate nuclei. Cholinergic blockade by atropine methylnitrate abolished bradycardia URs and CRs; whereas, beta-adrenergic blockade by propranolol did not diminish these responses. This indicates that autonomic mediation of the bradycardia responses consisted of increases in vagal tone. Alpha-adrenergic blockade by phentolamine abolished bradycardia URs, but not CRs, indicating that different central mechanisms mediated these responses. In a second experiment, one group of rabbits was injected with saline and another group was injected with phentolamine before each of six acquisition sessions. Both groups developed bradycardia CRs although phentolamine abolished the blood pressure URs and converted the cardiodecelerative UR to cardioacceleration.

Alpha-adrenergic blockade Beta-adrenergic blockade Atropine methylnitrate Classical conditioning
Intracranial stimulation Rabbit

THE PRESENT study used selective autonomic blockades to examine the relationship between heart rate CRs and URs during two classical conditioning experiments in which the US consisted of electrical stimulation of the hypothalamus or septal region. Previously, VanDercar, Elster and Schneiderman [12] examined heart rate and blood pressure changes in rabbits during a differential classical conditioning experiment in which short pulse-train stimulation of the septal region or hypothalamus was the US. The URs included an increase in blood pressure accompanied by bradycardia; CRs included bradycardia but no pressor response. Subsequently, Powell, Goldberg, Dauth, Schneiderman and Schneiderman [10] investigated autonomic mediation of the heart rate and blood pressure URs. By examining the cardiovascular changes occurring to the intracranial US under graded doses of selective autonomic blocking agents,

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they demonstrated that the bradycardia UR was mainly a reflex response to a sympathetically-induced increase in blood pressure. The present study also used autonomic blocking agents to interfere selectively with the autonomic innervation of the heart muscle and the arterioles controlling blood pressure, but this time in a conditioning experiment.

Autonomic innervation of the cardiovascular system can be pharmacologically classified as either alpha-adrenergic, beta-adrenergic or cholinergic on the basis of the blocking effects of drugs such as phentolamine, propranolol or atropine [13]. Atropine selectively antagonizes the parasympathetic innervation of the heart muscle; whereas, phentolamine and propranolol selectively block the sympathetic innervation of the arterioles (alpha-adrenergic) and heart muscle (beta-adrenergic), respectively. Since the bradycardia UR to short pulse-train stimulation of hypothalamus or septal region is a reflexive response to an increase in arterial pressure, whereas the bradycardia CR is not, the present study used the same autonomic blocking agents as Powell *et al.* in order to compare the autonomic mediation of heart rate CRs and URs; dosages chosen were those previously established in rabbits to be consistent with the pharmacological properties of the drug [10]. The present study also sought to determine whether bradycardia CRs would develop if the pressor UR, which reflexively elicits bradycardia, were abolished by injection of phentolamine prior to each acquisition session.

EXPERIMENT 1

Rabbits were classically conditioned, and then, on separate days given conditioning sessions following systemic injection of cholinergic or adrenergic blockades or saline. Since US alone as well as conditioning test trials were given, the procedure permitted a comparison of the sympathetic and parasympathetic influences mediating heart rate CRs and URs.

Method

Animals, surgery, and histology. The animals were 10 experimentally naive male and female albino rabbits (*Oryctolagus cuniculus*) weighing 2.9–3.5 kg at the time of surgery. Each rabbit was anesthetized with sodium pentobarbital (25–35 mg/kg, i.v.) and placed in a Neuman stereotaxic instrument. Our method for implanting electrodes and connecting them to the stimulation and recording equipment has previously been described [10,12]. In the present experiment four pairs of stainless steel side-by-side bipolar electrodes were implanted in each rabbit. Two pairs were aimed bilaterally at the lateral geniculate nuclei (LGN) as CS electrodes. Two additional pairs were aimed at the hypothalamus and septal region as potential US electrodes. The depth electrodes were constructed of size 00 Clay-Adams insect pins (shaft diameter 0.25 mm; tip diameter 0.03 mm) insulated except for 0.5 mm at the tip. Distance between the tips of each bipolar pair was approximately 0.5 mm. Stainless steel machine screws were also implanted for the purpose of epidural recording. They were imbedded bilaterally in the bone overlying the occipital cortex. A third stainless steel screw used as an indifferent recording electrode was implanted in the skull 10 mm anterior to bregma.

Following training, the animals were perfused with Formalin and the brains sectioned at 42 μ. Critical slices were photographed unstained using a modification of the procedure of Guzman, Alcaraz and Fernandez [3] to allow localization of electrode placements.

Apparatus. Each animal was restrained in a Plexiglas box with gross body and head movements further restricted by inserting the animal's head through an adjustable stock and clamping the ears to the front plate with a clamp covered with foam rubber. Two stainless steel safety pin electrodes were chronically inserted into the skin of the animal just behind the right front leg and just in front of the left hind leg in order to obtain heart rate recordings. Insertion was harmless and did not induce observable discomfort, attempts to dislodge the pins or infection.

Amplification and recording of the electrocorticogram and heart rate were provided by a Grass model 7 polygraph. A Grass model S8 stimulator in conjunction with two Grass stimulus isolation and two constant current units was used for stimulation of subcortical brain sites. Magnitude and shape of the stimulus pulses were assessed using a Fairchild dual-beam oscilloscope. Current intensities reported to the nearest 0.01 mA were accurate within ±5%.

A Western Union tape transmitter in conjunction with timers and relay circuitry was used to program the presentation of stimuli and recording of responses automatically. During each session the rabbit was enclosed in a sound attenuating, deactivated, refrigeration shell. A Grason-Stadler white noise generator provided masking noise. The polygraph, stimulator and programming equipment were located in one room; the animal in the refrigeration shell was located in an adjoining room that was lined with copper mesh to reduce electrical interference from recordings.

Procedure. One week after electrode implantation, heart rate URs to hypothalamic and septal region stimulations were examined in each animal. Monophasic rectangular pulses, each of 0.25 msec duration, were presented at 200 pulses per sec for 1.0 sec in an ascending then descending series of intensities at each septal region and hypothalamic electrode placement. Time between stimulations was 1.5 min. Stimulation at the electrode location and current intensity producing the largest heart rate decrease in the absence of struggling or gross movement was selected as the US for the subsequent conditioning sessions.

Following the US-alone session, rabbits received two days of adaptation to CS electrical stimulation of each LGN. The CS stimulation consisted of a 2.0 sec train of 15 monophasic pulses per sec with a pulse train duration of 0.5 msec and a current intensity of 0.2 mA. Fifty-eight trials were given per day, half at each LGN, at an intertrial interval of 1.0 min.

Differential classical conditioning was begun on the day following the second adaptation session. Each conditioning session consisted of 60 trials presented at an intertrial interval of 1.0 min. Of 30 CS^+ presentations (i.e., stimulation of one LGN) per day in acquisition, 27 presentations were immediately followed by the US, whereas three presentations of the CS^+ without the US (Trials 2, 30, 59) served as the CS^+ test trials. Of the 27 CS^- presentations (i.e., stimulation of the contralateral LGN) per day, three presentations (Trials 3, 28, 58) served as CS^- test trials. On three trials (Trials 1, 29, 60) only the US was presented. During CS^+ trials in which both the CS^+ and the US were presented, CS^+ was presented for 2.0 sec, the US was presented for 1.0 sec, and the CS–US interval was 2.0 sec.

Distribution of the CS^+ and CS^- trials within a session was random with the restriction that no more than two of one kind of CS occurred consecutively.

Fifteen minutes before the fourth acquisition session, each rabbit was given a subcutaneous injection of 3.0 cc physiological saline. On each subsequent day, the rabbit was also injected subcutaneously 15 min before the conditioning session and received either saline, atropine sulfate (20 mg/kg), atropine methylnitrate (20 mg/kg), phentolamine (5 mg/kg), or propranolol (5 mg/kg). On days in which phentolamine was injected, a second injection of phentolamine was administered 30 min after the beginning of the session. Drug days were always interspersed with saline days and each rabbit received each autonomic blocking agent during two separate sessions. If the animal showed evidence that it had not fully recovered from a previous drug session (e.g., assessed by baseline heart rate and/or debilitated conditioning), two or more saline sessions were interspersed to re-establish the spontaneous heart rate and conditioned heart rate baselines before another drug was injected.

Measurements. All heart rate measurements were based on five beat intervals measured to the nearest millimeter. Chart speed on test trials was 50 mm/min. On each test trial, baseline heart rate consisted of the mean 5 beat duration of two blocks of 5 heart beats occurring prior to stimulation onset. Responses were calculated by subtracting the duration of each block of 5 heart beats occurring after stimulation from the heart rate baseline (i.e., mean 5 beat duration before stimulation onset). Percentage heart rate changes from baseline were calculated on CS trials for five consecutive blocks of 5 beats and on US trials for three consecutive blocks of 5 beats. For statistical purposes the mean of 6 trials averaged over both drug sessions of each blocking agent was computed for each rabbit. The data for the saline control condition were computed using the mean of 6 trials averaged over a saline day immediately preceding and a saline day immediately following the phentolamine day.

Results

The US electrode placements for 3 rabbits were in the medial septal region and for 7 rabbits were in the hypothalamus. Hypothalamic placements were in ventromedial hypothalamus (n = 2), lateral hypothalamus (n = 2), lateral preoptic region, supramedullary area, and dorsomedial hypothalamus. Mean current intensity was 1.4 mA for the septal region placements and 0.5 mA for the hypothalamic placements. The form and magnitude of the heart rate URs differed little as a function of electrode placement. Location of the CS electrodes were in the LGN. All placements yielded comparable differential heart rate conditioning.

The effects of each blocking agent upon the heart rate baseline were assessed by examining the interbeat intervals of the 10 beats preceding the CS at 20, 40 and 60 min after administration of saline or each blocking agent. The interbeat interval measure was converted to beats per min. Mean heart rate baselines for each condition 20, 40 and 60 min after injection are shown in Fig. 1. Phentolamine, atropine sulfate and methylatropine increased, whereas propranolol decreased heart rate in relation to the saline baseline. An analysis of variance indicated that significant differences in baseline heart rate occurred as a function of treatments, $F(4,36) = 19.76$, $p<0.001$. Duncan range post-tests determined that the differences in baseline between the saline and all other drug conditions except for phentolamine ($p>0.05$) were significant ($p<0.01$). The analysis of variance indicated that reliable differences in the heart rate baseline did not occur as a function of time after injection, $F(2,14) = 2.34$, $p>0.05$, but a significant interaction between drug treatment and time after injection, $F(8,56) = 2.26$, $p<0.05$, apparently reflected the decreasing efficacy of atropine sulfate over time during sessions in which this drug was administered.

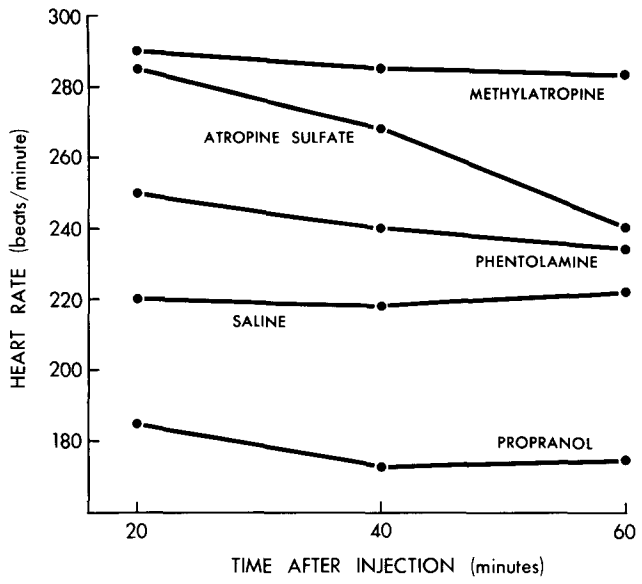

FIG. 1. Mean heart rate baselines for each condition 20, 40 and 60 min after administration of saline or 20 mg/kg methylatropine or atropine sulfate, 5 mg/kg phentolamine or 5 mg/kg propranolol.

Figure 2 shows the heart rate changes from baseline occurring during the 15 heart beats (3 blocks of 5 beats) following US alone stimulation during saline and drug sessions. The heart rate UR during saline injection sessions consisted of a decrease in rate which peaked during the second and third blocks of five heart beats following US onset. This heart rate decrease returned to baseline within 30 sec (not shown) after stimulation onset. The heart rate UR following propranolol injection was also a decrease, but this decrease peaked within the first block of five heart beats because the heart rate baseline in this condition was lower than in the saline condition (see Fig. 1). Phentolamine severely attenuated the heart rate decrease following US stimulation. The heart rate URs following injections of atropine sulfate or methylatropine consisted of a small increase in rate occurring in all animals.

An analysis of variance conducted upon heart rate percent changes from baseline for the three blocks of five beats following US onset indicated the presence of reliable differences among saline and drug treatments, $F(4,36) = 11.83$, $p<0.001$, blocks of five beats, $F(4,36) = 7.68$, $p<0.01$, and blocks by treatment interaction, $F(8,72) = 9.03$, $p<0.001$. Duncan range post-tests confirmed that heart rate URs under saline and propranolol did not differ reliably from each other ($p>0.05$), but the URs under both saline and propranolol differed reliably from URs

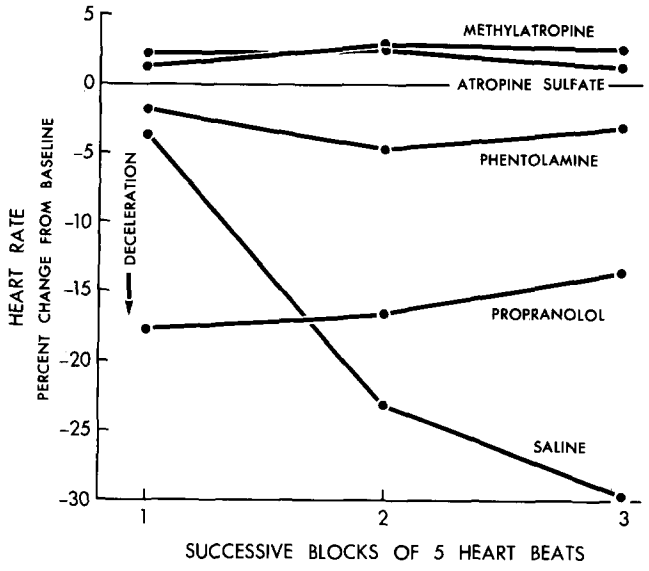

FIG. 2. Heart rate percent changes from baseline for 3 blocks of 5 successive heart beats following US stimulation under saline or 20 mg/kg methylatropine or atropine sulfate, 5 mg/kg phentolamine or 5 mg/kg propranolol.

under the other conditions. Because of the apparent differences in the topography of the heart rate URs in the saline and propranolol conditions (see Fig. 2), an analysis of variance was conducted upon the peak 5-beat magnitudes of post-minus pre-CS onset difference scores of the two conditions. This analysis failed to confirm the presence of significant differences in the peak magnitude of heart rate URs between the saline and propranolol conditions, $F(1,9) = 1.57$, $p>0.05$.

Figure 3 depicts the mean heart rate percent changes from baseline occurring to the CS^+ and CS^- during 5 successive blocks of 5 heart beats (25 beats) under the saline and each drug condition. The heart rate response to the CS^+ was clearly a decrease in rate except for the atropine sulfate and methylatropine conditions. Differential conditioning occurred in the saline, propranolol and phentolamine conditions, but not in the atropine sulfate or methylatropine conditions.

An analysis of variance conducted upon heart rate percent changes from baseline for the five blocks of heart beats following CS^+ onset confirmed that significant differences occurred among the saline and drug treatments, $F(4,36) = 7.24$, $p<0.001$. Significant differences were also obtained across blocks of 5 heart beats, $F(4,36) = 8.10$, $p<0.001$ and for the treatment by blocks interaction, $F(16,144) = 2.68$, $p<0.01$. Duncan range post-tests con-

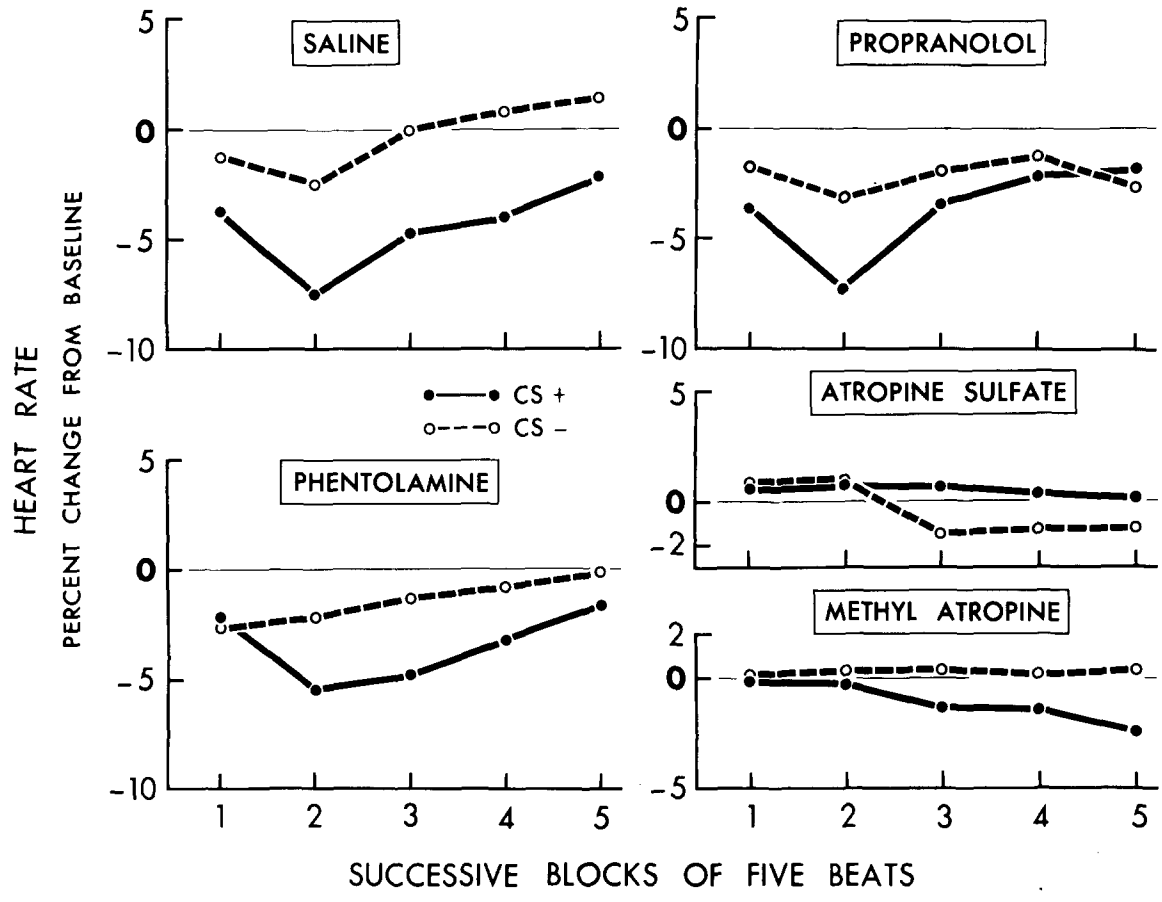

FIG. 3. Heart rate percent changes from baseline to CS^+ and CS^- for 5 successive blocks of 5 heart beats under saline or 20 mg/kg methylatropine or atropine sulfate, 5 mg/kg phentolamine or 5 mg/kg propranolol..

firmed that the magnitude of the CR^+ on saline days only differed reliably ($p<0.05$) from the CR^+ obtained on the atropine sulfate and methylatropine days. The CR^+ during saline, propranolol and phentolamine days did not significantly differ from one another. An analysis of variance conducted upon peak CR^+ minus CR^- differential conditioning score also confirmed that significant differences occurred among the saline and drug treatments, $F(4,36) = 3.54$, $p<0.02$, as well as for the blocks of 5 beats, $F(4,36) = 4.87$, $p<0.01$, and for the treatment by blocks interaction, $F(16,144) = 2.25$, $p<0.01$. Duncan range post-tests indicated that the saline condition differed reliably ($p<0.05$) from the other treatment conditions, that the propranolol and phentolamine conditions failed to differ from one another, and that the propranolol and phentolamine conditions differed reliably from the methylatropine and atropine sulfate conditions ($p<0.05$).

EXPERIMENT 2

In Experiment 1, rabbits were classically conditioned and then given systemic injections of selective autonomic blocking agents. The finding that methylatropine and atropine sulfate abolished the decelerative heart rate changes as both the CR and UR indicates that autonomic mediation of these responses occurred via an increase in vagal tone. The major observation of the study, however, was that the alpha-adrenergic blocking agent phentolamine abolished the heart rate UR, but did not abolish heart rate conditioning. This suggested that blood pressure increases may have mediated the UR but not the CR. In Experiment 2 the question was asked whether classically conditioned heart rate decreases in rabbits would develop if during acquisition, the blood pressure and bradycardia URs were abolished by systemic injections of phentolamine. Therefore, in one group of rabbits, acquisition sessions took place immediately after injection of phentolamine; whereas, in a second group of rabbits, acquisition sessions took place after injection of saline. In half of each group, blood pressure as well as heart rate was recorded during the course of the experiment.

Method

Animals. Twelve experimentally naive male albino rabbits (*Oryctolagus cunniculus*) weighing 2.5–3.5 kg at the time of surgery were used.

Surgery. Surgical implantation procedures were identical to those described in Experiment 1. In addition to electrode implantation, 6 animals in Experiment 2 were cannulated to permit chronic blood pressure recording. The cannulation procedure was carried out on the first day of acquisition in the following manner. Xylocaine was injected subcutaneously in the vicinity of the medial ear artery. The artery was then exposed and a 3-cm long polyethylene cannula was inserted into the artery and secured with surgical thread. An 18 ga blunted needle was inserted into the exposed end of the cannula, and the entire assembly was stoppered with the end of a 1 cc syringe and a removable rubber plug. The incision was closed and 0.2 cc of 100 mg/ml sodium heparin was injected into the cannula to flush it. In order to maintain cannula patency, this injection was repeated daily and supplemented with a 1.5 cc injection of 50 mg/ml heparin subcutaneously.

Apparatus. The apparatus for Experiment 2 was identical to that used for Experiment 1 with the following addition. A Statham P23 pressure transducer was mounted directly beside the Plexiglas restrainer box containing the rabbit. The tranducer was connected to the medial ear artery cannula by means of a twin site venotube inserted directly into the end of the blunted 18 ga needle.

Procedure. Prior to the first day of acquisition, rabbits were randomly assigned to one of two groups with 6 animals per group. The rabbits in the saline control group were each injected with 4 cc of saline divided into 2 injections per session. The animals in the phentolamine group were given 2 injections of 10 mg/kg per session.

Adaptation and acquisition procedures were identical to those described for Experiment 1 with the following exceptions. Following the second adaptation session, US intensity was readjusted to insure that the previously determined intensity was below the movement threshold. Changes were made for 4 animals that ranged from 0.05 to 0.10 mA. An additional set of test trials was presented daily prior to drug or saline administration during all 6 days of acquisition training. Subsequent to these pre-drug trials control animals were injected with 2 cc of saline, whereas experimental animals were injected with phentolamine. Ten minutes later acquisition trials recommenced with the first set of test trials as described in Experiment 1. Prior to the middle set of test trials (28, 29, 30), animals in the phentolamine group were given another full dose of phentolamine, while rabbits in the other group were given 2 cc of saline. After a 5 min rest period, test trials 28, 29 and 30 were given and conditioning continued. All other aspects of each acquisition session were identical to those in Experiment 1.

Measurement. As in Experiment 1, all heart rate measurements were based upon 5 beat intervals measured to the nearest millimeter. The prestimulation baseline was calculated as the mean of 2 blocks of 5 beat intervals and poststimulation measurements were based on 5 blocks of 5 beat intervals after stimulation onset. Heart rate percent changes from baseline were calculated as in Experiment 1.

Blood pressure topographies were obtained by comparing mean systemic blood pressure in millimeters of mercury (mm Hg) at the midpoint of each of the 2 pre- and 5 poststimulation blocks of 5 beat intervals. The magnitude of blood pressure changes after stimulation was computed by subtracting the blood pressure measurements for the averaged two prestimulus onset periods from those of each of the five poststimulation onset periods. A d.c. preamplifier channel of the polygraph used to record blood pressure was calibrated to permit pen deflections to be converted into mm Hg.

Results

For the rabbits in the saline group the US electrodes of 2 rabbits were in the medial septal region and the US electrodes of the other 4 rabbits were in ventromedial hypothalamus and the perifornical area. For the rabbits in the phentolamine group the US electrodes of 1 rabbit were in the medial septal region and those of a second rabbit were in the lateral septal region; US electrodes of 4 rabbits were in the ventromedial hypothalamus, lateral hypothalamus, dorsomedial hypothalamus and lateral preoptic region. The form and magnitude of heart rate and blood pressure URs differed little as a function of electrode placement. Location of the CS electrodes were either in or within 1.0 mm anterior to the LGN.

Figure 4 shows the topography of mean blood pressure

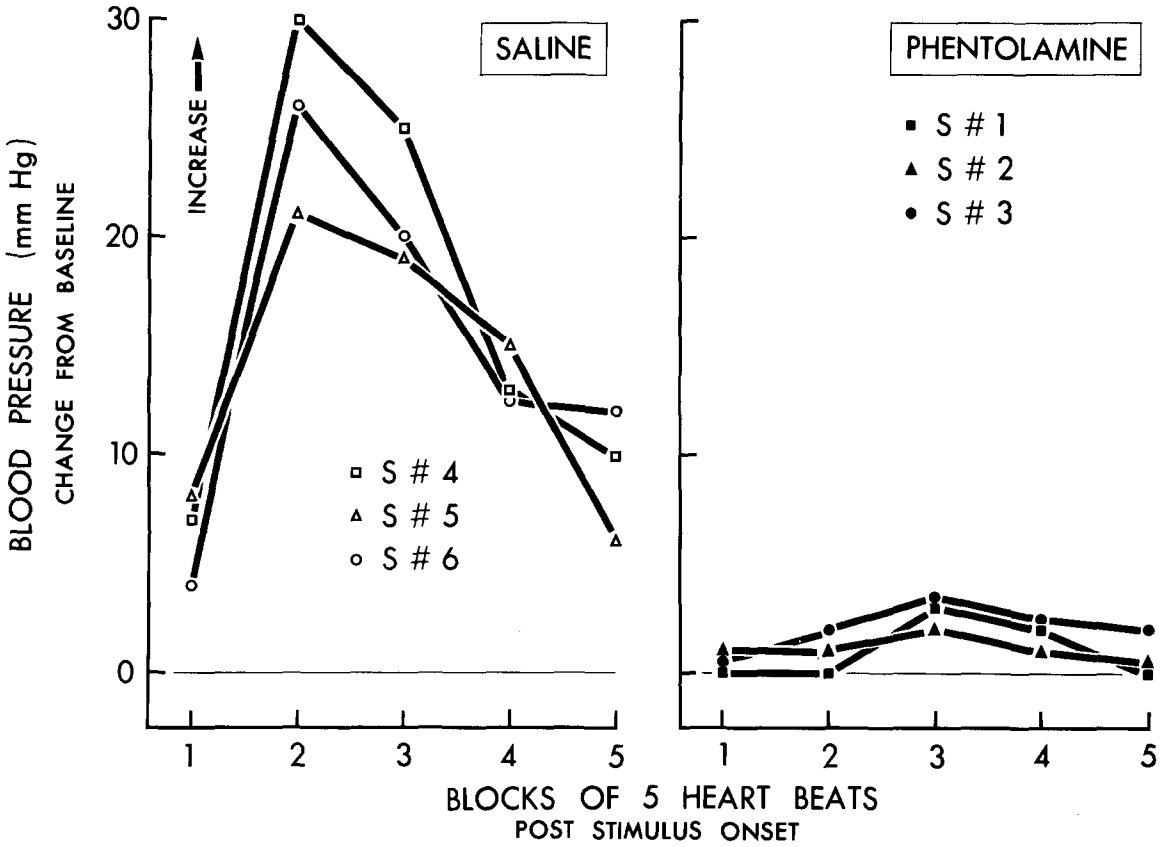

FIG. 4. Topography of mean blood pressure URs for 3 saline and 3 phentolamine injected rabbits.

URs for 3 control rabbits following injection of saline and for three experimental rabbits following injection of phentolamine. Peak mean blood pressure changes to US stimulation averaged 27 mm Hg and 2 mm Hg in the control and experimental groups, respectively. Comparable changes were observed for systolic and diastolic blood pressures, but were not subjected to statistical analyses. An analysis of variance conducted upon peak mean blood pressure URs indicated the presence of reliable differences, $F(1,4) = 108.49$, $p<0.001$, between saline and phentolamine injected animals. In contrast, an analysis of variance comparing peak mean blood pressure URs during the US alone trial preceding daily injections of saline or phentolamine did not confirm the presence of reliable differences between experimental and control animals $F(1,6) = 0.26$, $p>0.05$).

Figure 5 shows the response topographies of heart rate URs during acquisition sessions in the experimental and control groups following injections of phentolamine or saline. Injections of phentolamine abolished the cardiodecelerative UR, converting it into a small cardioacceleration. An analysis of variance conducted upon heart rate percent changes from baseline for the 5 blocks of 5 beats following US onset indicated the presence of reliable differences between saline and phentolamine injected animals, $F(1,10) = 11.80$, $p<0.01$.

Figure 6 depicts the mean heart rate percent changes from baseline occurring to the CS^+ and CS^- during 5 successive blocks of 5 heart beats during adaptation and differential conditioning sessions for animals injected with

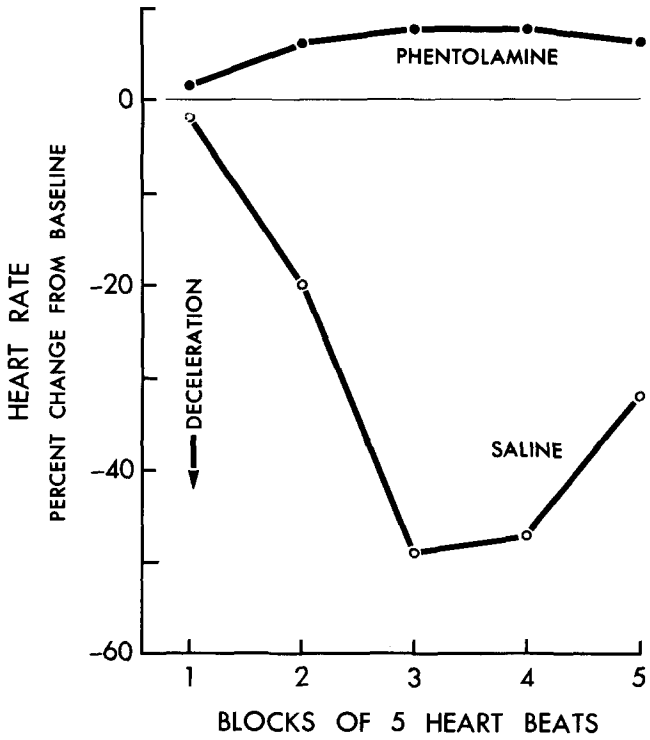

FIG. 5. Heart rate percent change from baseline for 5 blocks of 5 heart beats following the onset of US stimulation.

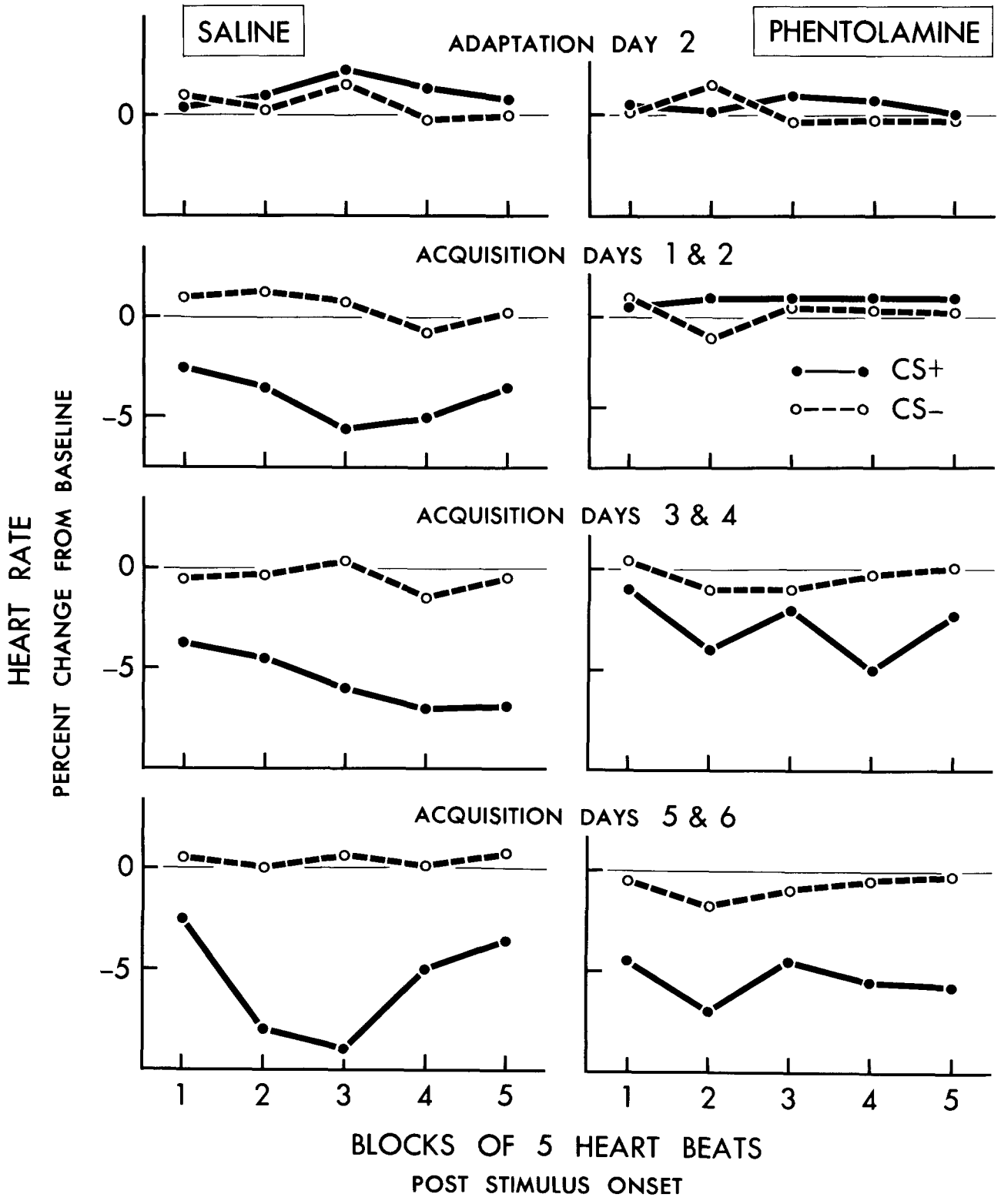

FIG. 6. Heart rate changes from baseline to the CS^+ and CS^- during adaptation and conditioning sessions. Changes are shown for 5 successive blocks of 5 heart beats following CS onset on test trials.

saline or phentolamine. Differential heart rate conditioning occurred more rapidly and peak magnitude was greater in the saline than in the phentolamine injected animals. Both groups, however, clearly developed differential conditioning. Evidence of blood pressure conditioning was observed in neither the phentolamine nor saline injected animals. A mean increase of less than 1 mm Hg. was recorded in each group with approximately the same number of blood pressure decreases as increases observed.

A repeated measures analysis of variance conducted upon the magnitude of heart rate percent changes during the 5 blocks of 5 heart beats following CS^+ in the phentolamine vs. saline injected groups revealed differences between conditions, $F(1,10) = 5.20$, $p<0.05$, and across days of acquisition, $F(2,10) = 14.86$, $p<0.001$, but not across blocks of 5 beats, $F(4,20) = 1.53$, $p>0.05$. A similar repeated measures analysis conducted upon the magnitude of CR^+ minus CR^- heart rate differential conditioning scores also confirmed that significant differences occurred between conditions, $F(2,10) = 4.94$, $p<0.05$, and across days of acquisition, $F(2,10) = 8.81$, $p<0.01$, but not across the 5 blocks of 5 heart beats, $F(4,20) = 1.85$, $p>0.05$.

A repeated measures analysis of variance conducted upon the magnitude of heart rate percent changes from baseline to CS^+ versus CS^- responses for days 5 and 6 of the conditioning sessions in the phentolamine injected group showed reliable differences between CR^+ and CR^-, $F(1,5) = 6.36$, $p<0.05$, indicating that differential conditioning was obtained in this group.

GENERAL DISCUSSION

Both atropine sulfate and atropine methylnitrate totally abolished the heart rate CRs and URs, whereas injections of propranolol had little influence upon these responses. This indicates that the CRs and URs were normally mediated by an increase in vagal tone. Moreover, the finding that the cardiodecelerative UR was converted to cardioacceleration by administration of atropine sulfate or atropine methylnitrate agrees with previous findings that using our parameter values of stimulation for the US, the cardiodecelerative UR normally masks a cardioaccelerative sympathetic component [10]. Since cardioaccelerative CRs were not observed after injections of methylatropine or atropine sulfate, it would appear that the heart rate UR but not the CR normally involved the sympathetic component. In contrast, during classical conditioning of human subjects using peripheral electric shock as a US, the normally observed cardiodecelerative CR has been reported to mask a sympathetic component that is only observed under atropine conditions [9].

The finding that injections of phentolamine which abolished the pressor UR also eliminated the cardiodecelerative UR is also consistent with previous findings in rabbits [10]. Since phentolamine is an alpha-adrenergic blocking agent that selectively blocks the tonic sympathetic vasoconstrictor tone of the arterioles without blockading the sympathetic innervation of the heart [8], the elimination of both the heart rate and blood pressure URs appears to have been due to the effect of the drug upon blood pressure. Because injections of phentolamine that abolished the bradycardia UR did not eliminate the bradycardia CR, the elimination of the bradycardia UR under phentolamine cannot be logically attributed to a change in the heart rate baseline. Instead, the present data indicate that the heart rate UR to short pulse-train duration stimulation of the hypothalamus or septal region in unanesthetized rabbits is a reflexive response to an increase in arterial blood pressure, whereas the heart rate CR is not.

The extent to which heart rate CRs occurred when heart rate URs were blocked pharmacologically can be evaluated by comparing the effects of atropine and phentolamine. Since the bradycardia URs and CRs were mediated by the vagus nerves, both were blocked by atropine sulfate and methylatropine. In contrast, phentolamine's influence upon bradycardia URs was indirect and occurred only because the heart rate UR was a reflexive response to an increase in blood pressure. In contrast, the heart rate CR was not influenced by phentolamine, because it was not dependent upon an increase in blood pressure, nor upon the occurrence of the bradycardia UR.

The first experiment of the present study indicated that bradycardia CRs were obtained under phentolamine once conditioning had already occurred. The second experiment indicated that bradycardia CRs can develop even if the UR constellation, including a blood pressure increase and bradycardia, is suppressed by phentolamine throughout conditioning trials. Our finding that cardiodecelerative CRs developed even when the pressor UR was suppressed indicates that the CR did not develop in response to a negative feedback pathway from the baroreceptors. It also appears unlikely that the bradycardia CR developed as a function of unspecified secondary effects of pharmacological blockade that produced tachycardia as the UR. Development of cardiodecelerative CRs in instances in which the UR was cardioaccelerative have been observed in the absence of pharmacological interventions in rabbits [6]. The present findings are also consistent with the report that bradycardia CRs in the rabbit can develop when the UR constellation of blood pressure increase and bradycardia is suppressed by systemic injection of 6-hydroxydopamine [7].

Recently, Schneiderman [11] has suggested that heart rate CRs are anticipatory adjustments which prepare organisms to either augment or cope with the effects of the US. In instances in which the US leads to increased muscular exertion and increased demand upon the cardiovascular system, the heart rate CR is likely to consist of an increase in rate. In contrast, during situations in which the US does not lead to muscular exertion, but in which the cardiovascular system becomes mobilized (e.g., the US leads to increases in systemic arterial blood pressure, cardiac output and perfusion of skeletal muscles), a cardiodecelerative CR would serve to decrease the stress placed upon the system. In the phentolamine condition of the second experiment, the US did not lead to movement nor to an increase in arterial blood pressure. This might suggest that the cardiodecelerative CR may not serve to compensate for sympathetically induced cardiovascular responses to the US in the nonmoving organism. However, blocking the blood pressure increase to the US does not abolish all sympathetically induced cardiovascular changes. While increases in arterial blood pressure primarily reflect vasoconstriction of cutaneous and splanchnic regions, they are often accompanied by vasodilation in skeletal muscle [2]. The vasodilation is not alpha adrenergic [1,5], so blockade of arterial blood pressure by phentolamine would not abolish it. The cardiodecelerative CR could therefore be adaptive in mitigating the overperfusion of vascular beds in skeletal muscle in the nonmoving organism. While perfusion of postural muscles

involved in an inhibition of movement might seem to be compromised by a cardiodecelerative CR, Hilton, Jeffries and Vrbova [4] have provided evidence that flow in the vascular beds of muscles involved in postural maintenance is high and relatively independent of control by the central nervous system.

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