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Peripheral and central muscarinic cholinergic blockade: Effects on Pavlovian conditioning

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Separate groups of rabbits were administered saline, atropine methyl nitrate, or atropine sulfate and differential Pavlovian conditioning was studied as a function of interstimulus intervals of 1, 2, 4, and 6 sec. Corneoretinal potential (CRP) and heart rate (HR) were assessed. Both methylatropine and atropine severely attenuated the HR conditioned response (CR) compared to saline control injections, although consistent, but small, bradycardiac CRs were obtained under both drug conditions. CRP CRs were almost completely abolished by the centrally acting atropine sulfate, and were moderately impaired by the peripherally acting methylatropine compared to animals treated with saline. The results suggest that, although central muscarinic cholinergic blockade severely interferes with Pavlovian conditioning, peripheral blockade also produces pronounced impairments in both autonomic and somatomotor response systems.

Concomitant conditioning of autonomic and somatic response systems have been the focus of recent studies in rabbits (e.g., Schneiderman, 1970), rodents (Black & de Toledo, 1972), and primates (Brady, 1975). Aside from ascertaining whether conditioning parameters differ for autonomic and somatic response systems, the rationale underlying these experiments is related to determining possible relationships between the two systems. We previously reported that cardiovascular and eyelid conditioning in the rabbit were correlated (Powell & Kazis, 1976). As eyelid acquisition proceeded, heart rate (HR) conditioned responses (CRs) became more accelerative and blood pressure CRs developed pressor characteristics. Based on these data, it was suggested that two separate and opposing processes might be responsible for the cardiovascular CRs that accompany classical conditioning of somatomotor responses. It was suggested that one process is related to attentional or orienting mechanisms, as previously discussed by several investigators (Graham & Clifton, 1966; Lacey & Lacey, 1974; Sokolov, 1963), and produces bradycardia. The second, an accelerative component, is probably associated with skeletal responding and becomes apparent only after attentional mechanisms become minimal. These latter changes are compatible with the characterization of the cardiac component of the "defense reflex" by Graham and Clifton (1966) and Sokolov (1963), and with the "cardiac-somatic linkage" interpretation of autonomic-somatic relationships as discussed by Obrist (Obrist, 1976) and others (Elliott, 1974; Schneiderman, 1970).

One technique for studying relationships between cardiovascular and somatomotor conditioning has been to produce pharmacological blockade of autonomic systems while assessing the acquisition of somatomotor responses. Kazis, Duncan, and Powell (1974) and Kazis, Milligan, and Powell (1973), for example, using both cholinergic and beta adrenergic blockade, demonstrated that both unconditioned and conditioned HR changes were jointly determined by the vagal and sympathetic inputs to the heart. Moreover, both beta adrenergic and muscarinic cholinergic blockade impaired the acquisition of the somatomotor eyeblink or corneoretinal potential (CRP) response. In these experiments the HR CR was attenuated and the HR discrimination did not occur in animals administered atropine. Propranolol, which is a beta adrenergic blocking agent, also produced decrements in HR CR magnitude, but significant HR discrimination occurred in subjects administered propranolol. It was suggested that perhaps a peripherally acting cholinergic blockade would be analogous to the action of the beta adrenergic agent propranolol, in that it would produce HR CR decrements but still permit the HR discrimination to occur. This hypothesis was based upon the assumption that the HR decrement is produced by peripheral interference with ACh muscarinic receptors, whereas the HR discrimination is dependent upon central ACh mechanisms and is thus blocked only by central cholinergic blockade. However, since the primary component of the HR CR is vagal, optimal conditioning parameters might be required to produce a response of sufficient magnitude to test this hypothesis. Thus, in the present experiment the conditioning parameters were optimized by utilizing longer interstimulus intervals (see Powell, Lipkin, & Milligan, 1974) while assessing both HR and CRP changes.

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METHOD

Subjects

Ninety-six male and female New Zealand albino rabbits were maintained on ad-lib food and water in an animal ward with a 7:00 a.m. to 7:00 p.m. light-dark cycle. All animals were run during the daylight portion of the L-D cycle.

Apparatus

The experimental compartments consisted of two ventilated sound-attenuated refrigerator shells. The animals were run in pairs while in Plexiglas restrainers with an adjustable rear panel and head stock. All data were recorded on a Grass Model 5 polygraph. Stainless steel safety pins were subcutaneously implanted on the right shoulder and left haunch for ECG recording. Stainless steel electrodes were inserted underneath the upper and lower eyelids and connected to the Grass polygraph for recording the CRP response. The definition and measurement of this response has been previously described (Powell, Schneiderman, Elster, & Jacobson, 1971). The CSs were 1-sec tones produced by Bud code practice oscillators. Standard BRS programming equipment scheduled the delivery of electric shock and started the programming equipment. The shock unconditioned stimulus (US) was produced by a BRS Model SG002 constant-current shocker. Stainless steel Michel wound clips served as shock electrodes. To minimize external noise, the recording and control equipment were located in a room adjacent to the experimental chambers.

Procedure

A differential classical conditioning procedure was used with a constant 90-sec intertrial interval. Two adaptation and eight acquisition sessions were employed. During adaptation only the 1,216- and 304-Hz tone CSs were presented; during acquisition one of the two tone CSs was paired with the shock US. The tones were 75-dB (SPL) intensity superimposed over 75-dB (SPL) white noise, both of which were presented through speakers situated 30 cm above the animal's head. CS onset was simultaneous with US offset. The US was a .5-sec-duration 5-mA-intensity ac shock train. A 1,216-Hz tone was the CS+ and a 304-Hz tone the CS− for half the animals; these conditions were reversed for the remaining half. There were 32 presentations of each tone per session, and, except during adaptation and test trials, the CS+ was always reinforced by the shock US, while the CS− was not. The CSs were administered in a random order, with the stipulation that 32 presentations of each be administered consecutively. Four interspersed presentations of each tone per session served as test trials, during which HR changes were recorded. The US was not administered during the CS+ test trials. HR measures were based upon 5-beat R-R intervals read to the nearest millimeter on the polygraph chart at a chart speed of 50 mm/sec. On each trial, baseline HR consisted of the mean 5-beat duration of the 10 beats immediately preceding CS onset. Post-CS measures were based upon the mean 5-beat duration of six successive blocks of 5 beats occurring after the onset of the CS. CS+ and CS− test trials occurred every eighth trial on a random basis, with the constraint that at least one of the four test trials for each tone occur during the first and last halves of the session. All HR changes were converted from R-R duration measures to beats per minute.

The 96 subjects were divided into three groups of 32 animals each, which received (a) atropine sulfate, (b) atropine methyl nitrate, or (c) saline as drug treatments. Atropine sulfate and atropine methyl nitrate were prepared as unit weight per milliliter physiological saline, so that a constant volume was injected equal in milliliter to the subject's weight in kilograms. The dosage of each drug was 25 mg/kg. These dosages are somewhat higher than those commonly used to produce muscarinic blockade in other species. However, the rabbit is markedly resistant to atropine-like drugs due to the presence of a liver enzyme, atropinesterase, which rapidly inactivates them (Ambache, 1955). All injections were made subcutaneously 15 min prior to the initiation of the session; however, no injections preceded adaptation sessions. Dose response functions were determined in a previous experiment (Kazis et al., 1973). Each drug group was further subdivided into four groups of eight subjects each, which received one of the four following interstimulus-interval (ISI) conditions: 1, 2, 4, or 6 sec.

All data were analyzed by repeated-measures analysis of variance; the rejection region adopted for all statistical comparisons was $p < .05$.

RESULTS

The results are shown in Figures 1 and 2. Figure 1 shows the average HR change over six blocks of 5 post-tone heart beats for CS+ and CS−. Pretone baseline HR and adaptation data are not shown. Although differential adaptation did not occur in the different drug groups, subjects administered atropine revealed faster baseline HR than the saline-treated subjects [$F(2,84) = 8.3$, $p < .01$] during acquisition. Mean baseline HR scores (beats/min) for the three drug groups were: atropine, 251; methylatropine, 243; saline, 191. As shown in Figure 1, the HR CR consisted of bradycardia for all groups of animals. However, the magnitude of the CR was considerably greater in animals administered saline compared to those administered either of the two drug conditions. In addition, animals administered saline consistently revealed greater magnitude HR CRs to the CS+ than to

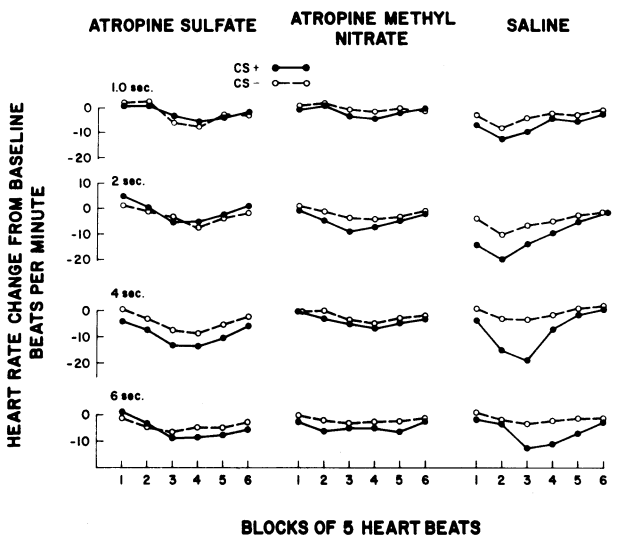

Figure 1. Mean post-CS heart rate change of rabbits administered atropine sulfate, atropine methyl nitrate, or saline and subjected to differential Pavlovian conditioning utilizing the interstimulus intervals shown. Data are shown as a function of six post-CS blocks of 5 heart beats and were averaged over eight acquisition sessions in which four measurements were made per session.

the CS–, whereas animals administered either atropine or methylatropine did not.

These data were analyzed by repeated-measures ANOVA, utilizing as repeated dimensions (a) sessions, (b) CS, and (c) blocks of five posttone heart beats. Significant drug [$F(2,84) = 14.1$, $p < .001$] and ISI [$F(3,84) = 4.6$, $p < .001$] effects were obtained, as well as significant Drug by Block [$F(10,420) = 3.46$, $p < .01$] and ISI by Block [$F(15,420) = 3.4$, $p < .01$] interactions. The triple interaction of Drug by ISI by Block was, however, not significant [$F(30,420) = 1.2$, $p > .10$]. The CS effect was significant [$F(1,84) = 10.6$, $p < .001$], and a significant CS by drug effect also occurred [$F(2,84) = 4.6$, $p < .01$]. The latter two findings suggested that the lack of discrimination in the drug-treated groups, compared to the saline-treated animals, was a reliable finding. In order to further test the reliability of this finding, discrimination scores were obtained by subtracting the CS– from CS+ scores for each animal. A subsequent repeated-measures ANOVA performed on these scores revealed a significant ISI [$F(2,84) = 6.5$, $p < .01$] and drug effect [$F(2,84) = 9.5$, $p < .001$] but an insignificant ISI by Drug interaction [$F(6,84) = .91$, $p > .10$]. Posttest comparisons of the HR discrimination scores revealed that, while the atropine sulfate and methylatropine groups were not significantly different from each other ($p > .10$), both differed significantly from the saline group ($p < .01$). This latter finding thus strongly suggests that, though a slight bradycardiac CR occurs in animals administered either atropine sulfate or methylatropine, HR discrimination is not reliable under either drug condition.

As noted above, baseline HR was significantly higher in the atropine groups than is the saline group. Thus, it is possible that the differences in HR CR magnitude between the groups were related to the elevated HR baseline, in accordance with the "law of initial values" (e.g., Kazis et al., 1973). To test for this possibility, the HR CRs were transformed to "autonomic lability scores" and reanalyzed (Lacey, 1956). This transformation results in corrected scores that possess a zero-level correlation with baseline HR. The results of an ANOVA of the corrected HR scores confirmed the findings previously described, in that none of the computed F values fell outside the established region of rejection. The conclusions, as described above, were thus confirmed by this analysis.

Corneoretinal potential changes are shown for animals in the 1-sec ISI conditions under saline, atropine methyl nitrate, and atropine sulfate conditions, respectively, in Figure 2. No CRP conditioning occurred at the longer ISIs, and the data are thus not shown. Data from adaptation sessions are also not shown in Figure 2. Animals administered saline revealed gradually increasing CRP CRs over the eight acquisition sessions. In addition, greater responding occurred to the CS+ than CS–. In comparison, animals administered atropine methyl

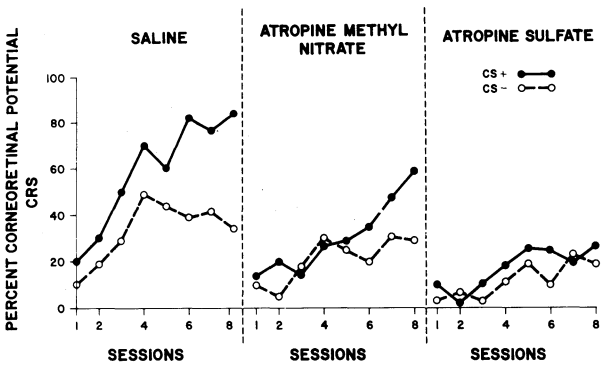

Figure 2. Percent corneoretinal potential CRs of rabbits administered saline, atropine sulfate, or atropine methyl nitrate and subjected to differential Pavlovian conditioning as shown.

nitrate revealed impaired acquisition of the CRP response, although reliable discrimination occurred. The centrally acting atropine sulfate, on the other hand, produced a severe impairment in CRP conditioning, as shown in the third panel of Figure 2. Little, if any, CRP discrimination occurred in this group. Analysis of variance of these data revealed a significant drug effect [$F(2,84) = 5.6$, $p < .01$], a significant ISI effect [$F(3,84) = 2.6$, $p < .01$], a significant CS effect [$F(1,84) = 3.9$, $p < .001$], and a significant sessions effect [$F(7,588) = 16.4$, $p < .001$]. Significant drug by CS [$F(2,84) = 3.6$, $p < .01$] and drug by sessions effects [$F(7,588) = 5.1$, $p < .01$] suggest that the differences obtained between the drug groups were reliable. Duncan posttests revealed that each of the groups differed significantly from each other in overall CRP responding ($p < .01$). This finding thus suggests that, although atropine sulfate almost completely abolished CRP conditioning, methylatropine also produced a significant impairment in CRP acquisition. A repeated-measures analysis of CRP discrimination scores in the 1-sec ISI condition revealed a significant drug effect [$F(2,21) = 5.64$, $p < .001$] and a significant Drug by Sessions interaction [$F(14,137) = 4.78$, $p < .001$]. Posttest comparisons showed that the saline group was significantly different from both drug groups ($p < .01$ and $p < .05$ for atropine and methylatropine groups, respectively). Moreover, the methylatropine CRP discrimination scores were also significantly larger than those of the atropine group ($p < .01$).

DISCUSSION

The results of the present experiment showed that the largest magnitude HR CRs occurred at 2- to 4-sec ISIs, while virtually no CRP CRs occurred at these intervals; moreover, CRP conditioning occurred only under the 1-sec ISI conditions. These differences in optimal ISIs for HR and CRP conditioning have been previously reported (Powell et al., 1974; Schneiderman, 1970).

Both atropine and methylatropine severely decreased the magnitude of the bradycardiac HR CR under all ISI conditions.

The lack of a significant ISI by Drug-Treatment interaction suggests that longer ISIs did not reliably produce larger HR CRs in the drug-treatment groups. Similarly, HR discrimination occurred in neither of the atropine-treated groups. Thus, the effects of central and peripheral muscarinic cholinergic blockade on HR conditioning were identical; that is, both discrimination and CR magnitude were severely attenuated. However, slight but significant bradycardiac CRs occurred under all ISI conditions in both drug groups, probably due to inhibition of sympathetic cardiac inputs. Support for this hypothesis was obtained in a prior study (Kazis et al., 1973), in which it was demonstrated that either atropine or propranolol attenuated but did not abolish HR CRs in the rabbit, whereas a double blockade utilizing both drugs completely abolished the response.

The effects of peripheral and central muscarinic blockade had differing effects on CRP conditioning. Central blockade with atropine sulfate almost completely abolished CRP responding, but a clear CRP discrimination occurred in subjects treated with methylatropine. This not unsurprising result confirms the dependence of CRP acquisition and discrimination on central nervous system mechanisms. However, subjects administered methylatropine revealed attenuated CRP conditioning compared to saline control subjects. This unsuspected finding may be due to some nonspecific effect of peripheral cholinergic blockade. However, it is also not inconsistent with the peripheral afferent feedback model of cardiovascular-behavior relationships (Lacey & Lacey, 1974). According to that model, blockade of peripheral feedback via specific visceral afferents interferes with cognitive processing and thus indirectly produces deficits in CRP acquisition. It is notable that peripheral muscarinic blockade has no effect on asymptotic nictitating membrane conditioning (Downs, Cardozo, Schneiderman, Yehle, Van Dercar, & Zwilling, 1972), but, as shown in the present study, severely retards CRP acquisition. The conditions under which such deficits occur clearly deserve further study.

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