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Learned Fear: A Cause of Arrhythmia Onset in the Presence of Digitalis[1]

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NATELSON, B. H., E. GROVER, N. A. CAGIN, J. E. OTTENWELLER AND W. N. TAPP. *Learned fear: A cause of arrhythmia onset in the presence of digitalis*. PHARMACOL BIOCHEM BEHAV 33(2) 431–434, 1989.—In earlier studies we have shown that guinea pigs exposed to signal-shock pairs develop digitalis toxicity earlier than control pigs on a test day when shocks are not delivered. Presenting subjects with signal-shock pairs is known to produce learned changes in autonomic tone thought to reflect fear. However, we were unable to find evidence of such changes in that model. A recent report extended our work on psychosomatic digitalis toxicity to the rabbit. Although that animal has been extensively used in studies of visceral learning, that study did not provide sufficient data to rigorously conclude that visceral learning had taken place. In this report, we show that rabbits which have learned that a signal accurately predicts the occurrence of shock develop digitalis-toxic arrhythmias more often during the signal and significantly earlier than other rabbits given prior exposure to equal numbers of signals and shocks, never explicitly paired. The use of this latter control group indicates that rabbits exposed to signal-shock pairs have learned to associate the signal with its consequences; independent evidence of learning exists in the fact that these rabbits showed a signal-locked bradycardia on their training day. However, bradycardia did not appear to be the mechanism for the early elicitation of digitalis toxicity on the test day when ouabain was infused during probes with the signal alone. These data may have clinical significance in their indication that factors in the external milieu can precipitate digitalis-toxic arrhythmias in individuals that would otherwise have no evidence of digitalis toxicity.

Rabbits Stress Arousal Emotions Forward conditioning Pseudoconditioning

THE digitalis glycosides have long been a mainstay of medical treatment for congestive heart failure, a syndrome that occurs in 1% of the population. In recent years, questions have been raised about the usefulness of digitalis in treating heart failure patients (1). However, several careful evaluations of this issue conclude that digitalis is effective in the patient with definite signs of heart failure (3,5). We have recently corroborated this assessment in a study of the effects of digitalis on lifespan and pathological signs of heart failure in hamsters with this syndrome (13). In addition, there is a dearth of effective inotropic agents available to treat heart failure. Therefore, it is clear that digitalis glycosides remain "a mainstay of therapy for congestive heart failure" (3).

The problem with digitalis is that there is a narrow zone between therapeutic efficacy and the potentially lethal toxic side effect of the drug—the production of cardiac arrhythmias. Because digitalis use is so common, it is important to define those variables which sensitize the individual to become digitalis toxic. For the most part, this analysis has been confined to variables within the internal milieu such as changes in concentrations of ions and hormones (7). But because autonomic neural activation also plays an important role in the genesis of digitalis toxicity (6), we reasoned that changes in the external milieu such as stress—a factor which alters autonomic neural tone—might also be a trigger for unanticipated digitalis toxicity. In a series of experiments using the guinea pig as subject (11), we collected evidence supporting the stress hypothesis which led to our suggestion that the physician might consider stress in evaluating the reasons for the development of digitalis toxicity in a previously stable patient.

In the model we used, guinea pigs were subjected to signalled shock (i.e., classical aversive conditioning). This paradigm is also called fear conditioning because other work has shown that the animals learn to associate the signal with the shock and show fear-like behavior accompanied by visceral changes indicative of fear when observed during probes with the signal alone (2). Guinea pigs exposed to fear conditioning developed digitalis toxicity significantly earlier than other animals given earlier exposure to either unsignalled shock or signal alone. However, with the conditioning parameters we employed, we were unable to

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demonstrate independent evidence of learning from heart rate data. Thus, we were interested to learn that Markgraf and Kapp had duplicated our model in the rabbit (10), an animal which has been extensively used in studies of visceral learning. These workers showed that rabbits exposed to fear conditioning and then infused with ouabain had episodes of digitalis-toxic arrhythmias more often during presentation of the signal than prior to it. However, because they did not employ a separate control group, it is not clear whether learning or some nonspecific factor related to signal and/or shock administration was responsible for the observed results. This paper reports our use of the rabbit model in a study designed to determine definitively whether the premature onset of digitalis toxicity has a learned component. The experiment used two groups of rabbits—a forward conditioned group in which signal and shock were explicitly paired and a pseudoconditioned group in which signal and shock were never paired. Our a priori hypothesis was that the occurrence of digitalis-toxic arrhythmias for the forward conditioned rabbits would begin more often than expected during the signal and/or would occur with shorter latencies than for the pseudoconditioned group.

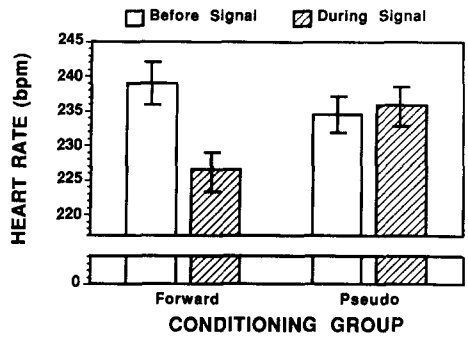

FIG. 1. Mean heart rates (± SEM) of forward conditioned (left most bars) and pseudoconditioned (right most bars) rabbits for the 10-sec periods before and the 10-sec periods of the signal. The data presented are averages based on data collected before and during every other signal on the training day when shock was delivered. Note that only the forward conditioned group shows a significant fear bradycardia to the signal.

METHOD

Animals

The subjects were 12 male albino New Zealand rabbits (Hare Marland, Hewitt, NJ) weighing about 1.5 kg. Rabbits were allowed to adapt to single cage housing with free access to food and water in our animal facility for at least 2 weeks prior to the experiment's start. During this time, each animal was handled three times a week for 1–2 min. Thereafter, when rabbits weighed about 2.25 kg, they were exposed to a handling/habituation period. During this period, rabbits were handled daily from Mondays through Fridays for 5–15 min and were then placed in the experimental apparatus for 1.5 to 2 hr per day. These sessions were done at least six times. On the third such session, loops of stainless steel surgical suture were placed subcutaneously over the left shoulder and the right haunch for ECG recording.

Apparatus

Animals were placed in a Plexiglas rabbit holder with adjustable headstock and backplate. Stainless steel dresshooks, slipped under the upper and lower eyelids of the left eye and held in place with a Velcro band around the head, served as electrodes for the presentation of the periorbital unconditioned stimulus. The restrainer and rabbit were then placed in a sound-attenuating Coulbourne chamber equipped with an exhaust fan and a sonalert. These methods for classical conditioning in the rabbit have been used extensively by others (8).

Training Procedures

Animals were randomly assigned to forward conditioning or pseudoconditioning groups (n = 6 in each). Forward conditioned rabbits were exposed to 20 signal-shock pairs. The signal was sounded for 10 sec and at its end, a 0.5 sec constant current 2.7 mA shock was delivered. Signal shock pairs were administered on a variable time schedule averaging 2.3 min. Pseudoconditioned rabbits were exposed to the same number of signals and shocks but these were not temporally contiguous. To prevent the possibility of the rabbit's learning any association between signal and shock, a minimum of 15 sec was programmed between either the start or end of the signal and shock occurrence. The temporal sequence of delivery of the signals was identical in the 2 groups. Stimuli were controlled by SKED software on a PDP-11 lab computer (16). The computer also controlled a Grass physiograph which recorded rabbit ECG for the 20 sec before and after each signal as well as during the 10 sec signal itself. Thus ECG was collected for 50 sec during each 2.3-min epoch.

Ouabain Probe

On the following day, the rabbit was handled and treated exactly as described above, except a 25-gauge needle was inserted into one of the marginal ear veins to allow ouabain infusion. Following placement of the restraining device within the sound-attenuating chamber, an intravenous ouabain infusion was begun at 2.2 μg/kg/min. Ouabain is a fast acting digitalis glycoside with a short duration of action. Twelve min later, the signal sequence was begun, but shock was never administered.

Data Analysis

Heart rate was hand counted in 5-sec bins for the 10 sec before and during every other signal on both experimental days. On ouabain probe days, each 50-sec physiograph record was evaluated for the presence of ventricular tachycardia (VT), i.e., 3 or more consecutive ventricular ectopic beats. To examine the effect of the signal on influencing the onset of digitalis toxicity, we compared the distributions of the times when rabbits in each group showed VT relative to the location of the signals. Finally, we determined the latency from the beginning of the ouabain infusion to onset of VT in the 2 groups as well as the heart rate of each rabbit immediately before it went into VT.

RESULTS

Evidence of Learning

Analysis of variance for repeated measures on heart rate data collected on the training day revealed a significant interaction between training group and heart rate before or during the signal, $F(1,10) = 14.1$, $p<0.01$. Figure 1 shows that heart rate for the pseudoconditioned rabbits is the same before the signal as it is during the signal. But forward conditioned rabbits show a small, but statistically significant decrease in heart rate during signal ($t_D = 4.7$, $p<0.01$). Panels A of Fig. 2 depict mean heart rate of the two training groups 12 min after the start of the ouabain infusion (i.e., immediately before the first signal was presented). The pseudoconditioned group has a significantly slower heart rate, $t(10) = 3.24$, $p<0.005$.

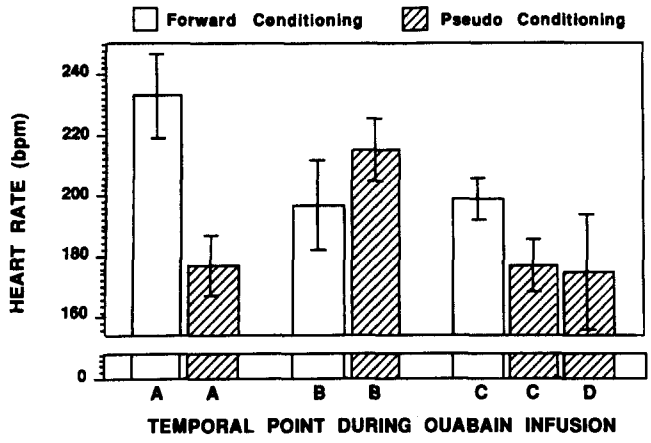

FIG. 2. Mean heart rates (±SEM) for rabbits during the ouabain infusion day when signals are delivered in the same temporal sequence as on the training day but shocks are never administered. Time A occurred 12 min after the start of the infusion prior to the delivery of the first signal. Time B was done prior to the delivery of the sixth signal; this was the last point in the experiments when all rabbits showed normal sinus rhythm on ECG. Time C was immediately before rabbits went into VT. Since the pseudoconditioned rabbits did so significantly later than the forward group, point D represents heart rate for the pseudoconditioned group at approximately the same time in the experiment as had been the case for the forward group.

Ventricular Arrhythmias

VT was detected *for the first time* in three out of six of the forward conditioned rabbits during a signal and in none of them in the 10-sec epoch prior to a signal. In contrast, VT was detected *for the first time* in three out of six of the pseudoconditioned group prior to the signal and in none of them during the signal ($p=0.05$, Fisher's exact test). As is evident from these data, three of the rabbits in each group showed their first run of VT at some time other than these 20 sec. Since recordings were made for an additional 30 sec, VT for these rabbits developed either before the 10-sec presignal period or after the 10-sec signal.

Because of this, a further evaluation of the ECGs of these animals was done to determine the location of a subsequent run of VT (i.e., where it first occurred in the pre-, during or postsignal sequence). Thus, for example, an animal that had developed VT for the first time before the presignal period but which was still in VT in the 10-sec presignal period was categorized as showing VT in the 10-sec presignal period for this analysis. As is evident, doing this was working against our seeing the initial run of VT during the signal. Using these criteria, VT was found for the first time in one additional forward conditioned rabbit before the signal, in 2 more during the signal and in none after the signal. For the pseudoconditioned rabbits, 2 more were found to be in VT before the signal, none during it and one went into VT after the signal.

When the data from this secondary classification were added to the data from the original analysis, finding a total of 5 of 6 rabbits in the forward conditioning group developing VT at only one of the three 10-sec periods evaluated was noted to be distinctly nonrandom ($p<0.002$, binomial test). Since this occurred during the signal, the data indicate that the tone elicited arrhythmogenesis. In the pseudoconditioned group, a total of 5 rabbits were in VT before the signal, none during the signal, and one after the signal. Since our analysis skewed the probability of finding abnormalities during the presignal period, this distribution is a random one. Pre- and post data were combined to provide a 2×2 table (i.e., pre and post compared to during signal and forward compared to pseudoconditioned) which was significant ($p=0.008$, Fisher's test). Latencies to onset of digitalis toxicity were also significantly different with forward conditioned rabbits going into VT earlier (40.2 ± 3.4 min SEM) than the pseudoconditioned group [48.3 ± 3.0 min; $t(10)=1.79$, $p<0.053$].

Relation of Heart Rate to Arrhythmia Onset

Panels C of Fig. 2 show heart rate just prior to VT for the 2 groups. The pseudoconditioned group had significantly slower heart rates at this time than the forward conditioned group, $t(10)=2.18$, $p<0.03$. However, this difference could be explained simply by the longer latencies until VT of the pseudoconditioned group. In the design used, longer latencies mean that more ouabain was infused and thus the differences in heart rate could simply be due to a greater digitalis-induced vagotonic effect in the group receiving more drug. Because of this possibility, we performed 2 additional statistical tests. First, we assessed heart rate in the pseudoconditioned rabbits in the 10-sec period before the twelfth presentation of the signal. We did this because, on the average, rabbits in the forward conditioned group developed VT during the twelfth 50-sec recording period. This allowed us to evaluate heart rate in the pseudoconditioned group after they had received approximately the same amount of ouabain as the forward-conditioned group. Figure 2 shows the data in panel D; no significant difference was found between heart rates of the pseudoconditioned done prior to the twelfth signal and heart rates of the forward conditioned group counted just before arrhythmia onset, $t(10)=1.27$. Another comparison of heart rates between the 2 groups was done in the 10-sec period before the sixth signal; that point was chosen because it was the last time when animals in both groups were arrhythmia free. Again, no significant difference was found between the two groups, $t(10)=1.0$; see Fig. 2, panels B. Finally, concerning the 5 forward conditioned rabbits that showed VT during a signal, heart rates, measured when the signal was on but immediately before the onset of VT, were 5% slower than they had been just before the signal began, $t(4)=3.09$, $p<0.05$. Thus, the small but significant conditioned heart rate slowing seen the day before is seen here too.

DISCUSSION

These data extend our earlier experimental observations to an animal—the rabbit—that has been extensively used in studies of visceral learning. Rabbits that were exposed to signal-shock pairs were different from other rabbits exposed to unrelated signals and shocks in that they developed VT more often during a probe with the signal and did so earlier—when they had received less digitalis. Forward-conditioned animals exposed to signal-shock pairs showed evidence of learning in the form of conditional bradycardia. Therefore, it appears that some learned change in physiological activity triggered digitalis-toxic ventricular arrhythmias and made arrhythmogenesis occur earlier than would have otherwise been the case.

We can exclude the possibility that the effects on digitalis toxicity are due to some nonspecific effects of electric shocks or to effects of the additional sensory stimuli of the signals because rabbits receiving the same number of noncontiguous signals and shocks were slower to develop digitalis-toxic arrhythmias. These controls were lacking in earlier studies (10,12) which therefore could not definitively attribute the sensitization to develop digitalis toxicity to an effect of learning. Since the appropriate pseudoconditioning control was used here, the effects noted cannot relate to the possibility that some nonspecific damage was done to the heart

as both groups received the same amount of shock. Further, since the experimental design used signals alone and no shocks on the ouabain infusion day, the psychological and physical components of the stimulus, which are so often inextricably intertwined in other studies of the effects of stress, are clearly separated. The only difference in the treatment of the 2 groups was whether the signal effectively predicted shock. Therefore, the learned response to that signal *must* be responsible for producing early onset of digitalis toxicity in the forward conditioned group.

The rabbit model has one important advantage over our previous guinea pig model. In that model, we were never able to detect any independent measure of learning other than early onset of digitalis toxicity in guinea pigs subjected to signal-shock pairs. In contrast, the rabbit is an animal that has been extensively used in studies of visceral learning (8). As expected, forward-conditioning rabbits showed a significant, although small, bradycardia during the signal. The fact that this signal-locked bradycardia did not occur in the pseudoconditioned group indicates that the forward- or classically-conditioned rabbit had learned that the signal reliably indicated the delivery of shock rather than a nonspecific response to the signal itself. In fact, the signal took on fear-producing qualities in the forward-conditioned rabbits, as evidenced by bradycardia—a cardiac rate change which occurs when feral rabbits are frightened by confrontation with humans (15).

Learning can also be invoked to explain the differences in heart rates seen prior to the presentation of the first signal probe on the ouabain infusion day. In simple conditioning situations, the animal will become conditioned to the cue that best predicts the shock. In the case of the pseudoconditioned rabbit, the best predictor of shock is their being in the experimental chamber. This phenomenon is well known as contextual conditioning (9). However, a temporally contiguous signal, such as the forward-conditioned group received, is a better predictor of shock and thus animals will learn to tend to it and not to less relevant cues in the environment (9). Thus, the pseudoconditioned group responded to the context more than the forward-conditioned group as evidenced by their having significantly lower heart rates than the forward-conditioned group. Such differences in heart rates have been noted in similar conditions by others (4).

The simplest explanation of the sensitization seen in this experiment is that the signal produces enough bradycardia to allow a digitalis-induced irritable focus in the ventricle to become the dominant pacemaker. That this relation holds is a well known fact which can be clinically useful in assessing a patient for covert digitalis toxicity (7). However, this explanation does not appear to explain the data presented here. No significant differences were found at similar time points between the 2 groups, and, consonant with its longer latency until VT, the pseudoconditioned group had a significantly slower heart rate immediately before VT was first seen than the forward group.

Although the drug's vagomimetic effect on rate does not appear to be responsible for the sensitization seen, the fact that the sensitization can be blocked by quaternary atropine salts which do not enter the brain (10,11) suggests that some peripheral muscarinic site is activated to produce this psychologically-induced precipitation of digitalis toxicity. Although the exact location of that site will require further research, the data reported here make it clear that psychologically relevant stimuli can produce digitalis toxicity in an individual with no previous evidence of this problem.

A question concerning animal research is how germane such studies are to human illness. The final link between animal research and human disease can rarely be made in studies of stress for ethical reasons. But if, as may be the case, these animal data can be extrapolated to patients taking digitalis, it may mean that stress in general and intensely arousing stimuli in particular must be considered by the physician who is suddenly presented with a patient taking digitalis whose ECG was previously normal and suddenly shows signs of digitalis toxic arrhythmias. Retrospective studies done on human victims of sudden cardiac death substantiate the idea that acute stress contributes to risk in the patient with structural if not necessarily symptomatic heart disease (14,17). We believe that the model we are developing can be useful in understanding how stress increases the risk of arrhythmogenesis and what can be done to counteract this.

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