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A Role for Environmental Factors in the Production of Digitalis Toxicity[1]

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NATELSON, B. H., S. L. HOFFMAN AND N. A. CAGIN. *A role for environmental factors in the production of digitalis toxicity*. PHARMAC. BIOCHEM. BEHAV. 12(2)235–237, 1980.—The purpose of our study was to learn whether changes in the external milieu could affect the lethality of ouabain. We found that guinea pigs experiencing restraint stress for the first time showed a greater susceptibility to the lethal effects of ouabain (175 μg/kg IP) than non-stressed controls. Adaptation to the restraint procedure abolished this sensitization. This effect related to repeated experience with restraint and not to repeated human handling because repeatedly handled guinea pigs still showed sensitization to the lethal effect of ouabain (200 μg/kg IP) when restrained for the first time. These data indicate that environmental factors will have to be considered in addition to changes in the internal milieu when trying to explain individual differences in sensitivity to toxicity while taking constant doses of digitalis.

Arrhythmias Guinea pigs Digitalis toxicity Ouabain Stress

THE narrow zone between therapeutic efficacy and potentially lethal toxicity in the digitalis-treated patient makes it imperative to define those factors which increase the likelihood that toxicity will develop. Serum levels of digitalis are somewhat helpful in labeling a group of patients that are or may soon become digitalis-toxic. However, the wealth of studies correlating clinical state with serum level of the drug has made it clear that at similar serum levels and dose regimens, one patient may be fine while another is severely toxic [10]. This overlap is related to the variety of factors that can influence a person's response to cardiac glycosides. For the most part, emphasis has been placed on metabolic factors. Thus, doses of digitalis which are not of themselves toxic become so when the patient has impaired renal function, thyroid disease or abnormal concentrations of potassium, sodium, calcium or magnesium [10]. Additional animal work has demonstrated that autonomic nervous activity also can affect the likelihood that digitalis-toxicity will develop in the previously non-toxic subject. This has been shown in experiments in which more digitalis is required to produce ventricular arrhythmias in the spinal-sectioned than in the intact animal [3] and in other work in which a dose of ouabain, not producing arrhythmias, does so when sympathetic activation is superimposed by electrophysiological techniques [2]. Based on this evidence, we reasoned that psychological stress, a known activator of the autonomic nervous system [5,8], might affect the dose of ouabain producing a lethal outcome. Moreover, if this were true, it would mean that physicians might have to extend their search for causes of digitalis-toxicity beyond the internal milieu [10] to include environmental and psychological factors. Recently, we have shown that these factors indeed can shift an individual animal's response to a fixed dose of the drug [6]. We now report shifts in the lethality of a rapidly acting digitalis glycoside, ouabain, following restraint—with increases or decreases in lethality relating to the animal's experience with the stressor.

METHOD

Animals

These experiments were performed on adult male Hartley albino guinea pigs, weighing at least 800 g. The animals were received from the supplier (Camm Research Institute) and were allowed 6–7 days to acclimate to being individually housed in suspended wire cages with ad lib access to food and water.

Procedure

Stress experiments of two types—acute and chronic—

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were performed. In the acute studies, animals were removed from their home cage, weighed, and then given an intraperitoneal injection of ouabain octahydrate (Eli Lilly and Co., Indianapolis, IN). One dose, either 150, 175, 200 or 225 μg/kg, was used. They were then placed back in their home cage for 45 min. Following this, the animals were again removed from their home cages and were than randomly placed into one of three treatment conditions. In the *home cage* condition, each animal was returned to its home cage with food or water removed. In the *novel cage* condition, each animal was placed into an opaque plastic cage (23×43×14 cm) prepared with laboratory bedding and a wire grid top. In the *restraint* condition, each animal was placed on a restraint board prepared to allow us to snare snugly the four paws with leather loops and to limit further the animal's ability to move by a 17 cm leather flap across its body.

Two chronic stress studies were performed (Chronic A and Chronic B). In the first of these (Chronic A), following the acclimatization period, animals were removed from their home cages, weighed, given an intraperitoneal injection of saline and then returned to their home cages. Forty-five minutes later, animals were again removed from their home cages and then were randomly assigned to either a *home cage* group or a *restraint* group. The *home cage* animals were returned to their home cages after removing food and water, while the restraint animals were subjected to restraint for 4–5 hr. This procedure was repeated for five additional weekdays. However, on the last of these sessions, the intraperitoneal injection was of ouabain, not saline. *Home cage* and *restraint* animals received the same ouabain dose, either 175, 200 or 225 μg/kg. In the second chronic stress experiment (Chronic B), following the intraperitoneal injection of saline and the 45 min waiting period, animals were again handled but then all were replaced in their home cages with no food or water available. This 5–6 hr procedure was repeated over the next 4 subsequent weekdays. On the following day, animals were injected with ouabain (200 μg/kg) instead of saline, and then after the 45 min waiting period, some were returned to their home cages while the others were subjected to restraint for the first time.

On ouabain injection days, following the 45 min waiting period, guinea pigs were checked for either spontaneous movement, response to a breath of air or response to a corneal touch every 5 min for 2 hr and then every 10 min thereafter until a maximum of 4–5 hr had passed. The time to death—the point at which the corneal reflex was absent—was recorded.

Mortality data were analyzed for significance by Fisher's exact test. The comparison across experiments regarding the frequency of early death was done by a chi-square test.

RESULTS

As shown in Table 1, only 9% of 11 guinea pigs given 175 μg/kg of ouabain and returned to their home cage died while the mortality rate was 50% among 14 animals that were acutely restrained ($p<0.05$). However, among 8 animals given ouabain 175 μg/kg and chronically handled and restrained, none died (significantly different from acute restraint, $p<0.03$). There was also no mortality at this dose among 8 pigs that were repeatedly handled and returned to their home cage. Because significant differences were not found between home cage and novel cage groups at any of the doses in the acute experiments, the novel cage condition was omitted in the chronic experiments. So too was the lowest dosage of ouabain (150 μg/kg) because neither control nor restrained animals died following its administration.

TABLE 1
MORTALITY RESULTING FROM OUABAIN

Dose		Home cage	Restraint
175μg/kg	Acute	9% (n=11)*	50% (n=14)†
	Chronic A	0% (n=8)	0% (n=8)§
200μg/kg	Acute	45% (n=9)	56% (n=9)
	Chronic A	17% (n=6)	14% (n=7)§
	Chronic B	27% (n=22)†	67% (n=24)‡¶

*Significantly less home cage than restraint deaths, $p<0.05$.
†Significantly less home cage than restraint deaths, $p<0.01$.
‡Significantly more mortality than Chronic A restraint at this dose, $p<0.03$.
§Chronic A group was restrained in every session, see text.
¶Chronic B group was restrained only in last session, see text.

The difference in mortality between the home cage and acute restraint group was lost when ouabain was given at higher doses. However, similar to those animals given 175 μg/kg of ouabain, when 200 μg/kg of the drug was administered, the chronic restraint group (Chronic A) tended to have a lower mortality (14% of 7 animals) than did the acute restraint group (56% of 9 animals). The mortality of this chronic restraint group (Chronic A) was also significantly ($p<0.03$) less than that of the group (67% of 24 animals) that was repeatedly handled but restrained only during the last session (Chronic B). In addition, the Chronic B restraint group suffered a significantly ($p<0.01$) higher mortality than did its control group which was repeatedly handled but never restrained.

Ouabain, at 225 μg/kg proved to be fatal for most guinea pigs. Thus, 80% of 5 home cage and 75% of 4 restraint guinea pigs died during the acute experiments; in the Chronic A group, 91% of 11 home cage and 84% of 12 restraint guinea pigs died. Interestingly, 4 of the Chronic A restraint guinea pigs died in their home cage during the 45 min post-injection period; none of the non-restraint home cage animals died early; and none of the pigs that died after receiving 225 μg/kg of ouabain in the acute experiments died early. This distribution of early mortality was non-random ($p<0.02$). The time to death was otherwise independent both of the dose of ouabain and the treatment condition: on the average, if it occurred, it was seen 1.5 hr after the ouabain injection.

DISCUSSION

These experiments demonstrate that 175 μg/kg of ouabain is more likely to be lethal when given to guinea pigs subjected to the stress of acute restraint than when given to unrestrained animals. This effect is related to restraint itself and not merely to our placing the guinea pigs in a novel environment, as evidenced by the fact that no differences in mortality were found between ouabain-treated animals which were returned to the home cage or placed into a novel cage environment. This finding of stress-induced sensitization to the lethal side-effects of ouabain supports our earlier report in which arrhythmias were found to occur significantly more often during a signal which had previously been correlated with shock when compared to a control group in

which the signal did not predict shock [6]. Our reason for turning to the restraint model here was our observation that control animals in our earlier paradigm also developed arrhythmias—albeit at a lower overall frequency. This suggested to us that our procedure which employed moderate restraint was in itself stressful and thus capable of diminishing the effect we had noted. The experiment reported here supports this notion.

Our next finding was that repeated exposure to restraint produces both sensitization and protection to stress-induced digitalis-toxicity. The sensitization seen was somewhat subtle. At the dose of 225 μg/kg of ouabain, a subgroup of guinea pigs, that had been repeatedly restrained, died during the 45 min postinjection control period during which the animal was in its home cage. No other subgroup of animals treated with this same ouabain dosage, which was enough to be lethal to almost all of them, died during this time period. We do not know the reason for the sensitization but have noted it before [6].

Protection was noted in that repeatedly restrained animals did not consistently die at doses of 175 μg/kg or 200 μg/kg, whereas in the acute experiments, they did so at 175 μg/kg. Similarly, Skinner *et al.* [9] have shown that repeated confrontation with a psychological stressor reduces the probability of its producing arrhythmias during experimental myocardial ischemia. Our next question was whether this protection was merely due to repeated handling and saline injection or due to the repeated exposure to restraint. The data suggested that repeated handling and saline injection alone contributed little to the protection because significant differences in mortality were not found for acutely handled guinea pigs when compared to repeatedly handled ones. However, because a tendency did exist for chronically handled animals to show less mortality than acutely handled animals, the third experiment was performed. Here pigs were repeatedly handled and given IP saline injections and only on the ouabain-injection day were half of the animals put into restraint for the first time. Despite the repeated handling and injection procedure, the stress of acute restraint produced as many deaths as acute restraint without repeated exposure to human handling. Thus, the stress implicit in repeated handling and IP injection of saline did not protect guinea pigs from the greater stress of acute restraint.

The questions that remain relate to the reasons for the sensitization to ouabain's lethal effect produced by the stress of a first exposure to restraint and the protection offered by repeated exposure to restraint. The literature offers some possibilities: in anesthetized animals, digitalis-induced arrhythmias are preceded in time by a massive autonomic nervous discharge which plays upon the heart [3] and upon the adrenal medulla [4]. Similarly, unanesthetized, restraint-stressed animals show striking changes in autonomic activity as manifested by long-lived heart rate increases [7] and by large increases in plasma levels of norepinephrine and epinephrine [8]. We believe that less ouabain was needed to produce lethal consequences in acutely restrained guinea pigs because of an additive effect of restraint stress and ouabain toxicity on autonomic activation. Furthermore, we believe that repeated exposure to restraint allowed habituation of restraint-induced autonomic arousal such that the additive effect on autonomic activation was lost. We have seen such habituation during other stresses in levels of glucose and renin whose release into the blood relates to changes in autonomic activity [5]. Whether or not this attenuation of visceral responsiveness extends to more pertinent blood-borne elements such as epinephrine and norepinephrine remains a question for further research.

The work reported here can be viewed as an outgrowth of the seminal work of Bajusz and Selye [1] who showed that the combination of fluorocortisol and sodium acetate may or may not produce cardiac disease depending on the amount of stress that was superimposed. Since the combinations of drugs used in those early studies were seldom prescribed, the pertinence of the work to clinical pharmacology was unclear. This is not the case in this report in which the toxicity of a cardiac glycoside was studied. Our data indicate that environmental factors will have to be considered in addition to changes in the internal milieu when trying to explain individual differences in sensitivity to toxicity while taking constant doses of digitalis.

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