On the cumulation of digitoxin and digitoxigenin in isolated heart muscle preparations

In recent investigations it could be demonstrated that after presaturation of isolated atria with [^{3}H]-labelled cardenolides the total tissue concentration of these drugs declines very slowly during wash-out (Kuschinsky, Lüllmann & Zwieten, 1968a,b). The positive inotropic action of these drugs, however, disappears far more rapidly on wash-out than might be expected from the decline in tissue concentration (Lüllmann, Weber & Zwieten, 1969). It seemed to us of interest to know whether in isolated heart muscle preparations cardenolides showed cumulative effects when, after wash-out of an initial effect, the same drug was applied a second time in the same concentration. The second dose was administered after wash-out in such manner that approximately half of the initial tissue concentration was left, although at that moment the positive inotropic action had already disappeared. Although digitoxin is known to accumulate *in vivo* as a result of slow renal elimination, its cumulative effect in isolated heart muscle of mammalian species has not yet been studied (Kuschinsky, 1968). Isolated atria, dissected from guinea-pigs of either sex (weight 250–350 g) (Hoditz & Lüllmann, 1964) were suspended in oxygenated Tyrode solution, containing 1·2 m-equiv Ca^{2+}/litre. The volume of the organ bath was 20 ml, its temperature was kept at 30°. The atria were stimulated electrically by means of rectangular pulses (duration 3 ms, frequency 180/min). The contractions were recorded continuously via a strain gauge and a Hellige type 86HE-t recorder. Digitoxin or digitoxigenin were injected into the organ bath after 30 min of equilibration. The final concentration was 1×10^{-7} g/ml for both drugs (i.e. digitoxin $1{\cdot}3 \times 10^{-7}$M and digitoxigenin $2{\cdot}7 \times 10^{-7}$M). The increase in contractile force was established and expressed as percentage of the initial value. The maximal effect was reached about 15–30 min after drug administration. Subsequently, 60 min after application of the cardenolide the medium was rapidly replaced by cardenolide-free Tyrode solution. After a wash-out period of 30 min, a second dose of digitoxin or digitoxigenin was administered in such manner that the same final bath concentration was reached as after the first application. Again, the inotropic effect was determined and compared with the effect of the first dose. In separate control experiments, the effects of digitoxin and its aglycone were studied in atria which had been equilibrated for 120 min, i.e. the same period as required until in the first series of organs the second amount was administered. These control experiments were designed to establish whether after a longer period of equilibration (120 instead of 30 min) the inotropic effect of the cardenolides would be different, since the contractile force of isolated atria usually decreases upon prolonged incubation.

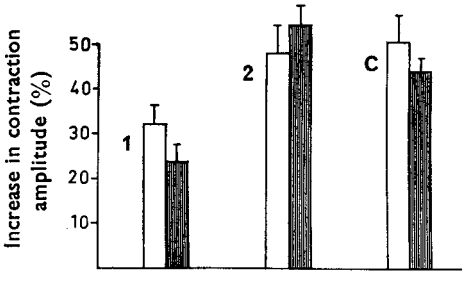

FIG. 1. Positive inotropic effect of digitoxin (open columns) and digitoxigenin (hatched columns) after 30 min of equilibration (first application) and after wash-out of the first dose for 30 min (second application). C = effects of both cardenolides (1×10^{-7} g/ml) after 120 min of equilibration (control experiments).

The results of our experiments are shown in Fig. 1. After an equilibration period of 30 min digitoxin and its genin caused positive inotropic effects of 32·3 ± 3·8% (n = 23) and 24·0 ± 3·8% (n = 13), respectively (mean ±s.e.). After the second application of digitoxin and its genin in the same organ after wash-out as described above, the contraction amplitude increased by 48·3 ± 6% (n = 17) and 54·3 ± 4·5% (n = 11) respectively. For both drugs, these effects are significantly larger than those of the first dose ($P < 0{\cdot}01$, Student's *t*-test). If, however, the first amount of digitoxin or its genin was administered after an equilibration period of 120 min, as in the control experiments, the positive inotropic effects were 50·6 ± 5·7% (n = 14) and 48·3 ± 6·0% (n = 17). For both drugs this increase in contractile force was significantly higher ($P < 0{\cdot}01$) than that observed after an equilibration period of 30 min. On the other hand, no difference existed ($P > 0{\cdot}05$) between the effect of a second application (120 min after the beginning of the experiments) and that of the cardenolides administered after 120 min without a first amount, as in the control experiments. Obviously, the mere fact that the equilibration period has been extended from 30 until 120 min causes an increased inotropic response to digitoxin or its aglycone. Therefore, no cumulative effect occurs if two subsequent doses separated by an interval of 30 min of wash-out are applied. The increased response to the second application is only due to the prolonged incubation of the isolated organ. As would be expected, the contraction amplitude was lower after 120 min of incubation than after 30 min. Accordingly, the relative increase will be larger after 120 min than after 30 min of incubation. Concomitantly, there exists no evidence whatsoever for a cumulative effect of either digitoxin or its aglycone in isolated heart muscle under the given experimental conditions. After wash-out for 30 min the tissue concentration of digitoxin is still about 60% of the initial value, whereas for digitoxigenin 53% is left in the isolated atrial tissue (Kuschinsky & others, 1968a,b). In spite of the quite considerable concentration of cardenolide left in the tissue, the newly added amount of either drug did not give rise to an increased pharmacological effect.

These results are in accordance with those of Lullmann & others (1969) which demonstrated that the positive inotropic effect of digitoxin disappeared after 15–25 min of wash-out, whereas for digitoxigenin only 5 min were required. Obviously, no cumulative effect occurs in isolated atria on wash-out because the positive inotropic effect of the cardenolides declines far more rapidly than the total tissue concentration of the drug. The present experiments once more confirm that most of the digitoxin (or its aglycone) accumulated by heart muscle is bound in a non-specific manner, and is of no importance to the inotropic effect.

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