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The influence of hexobarbitone on calcium ion movements in isolated heart muscle

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In beating atria (frequency 180/min) sodium hexobarbitone ($7{\cdot}8 \times 10^{-4}$M) inhibited the rate of uptake of $^{45}Ca^{2+}$ from the medium without influencing the total calcium content of the tissues. Concomitantly, the so-called exchangeable calcium fraction was diminished although not at equilbrium. In resting left auricles the barbiturate did not affect $^{45}Ca^{+}$-uptake or the exchangeable calcium fraction. It seems likely that the barbiturate reduces membrane permeability towards Ca^{2+} during excitation without influencing the permeability at rest.

Until now, relatively few studies of the influence of barbiturates on ionic fluxes in heart muscle have been published. Klaus and Lüllmann (1961) demonstrated an impaired K^{+}-efflux as a result of treatment with sodium hexobarbitone. Similarly, the efflux of $^{86}Rb^{+}$ from atrial tissue was also inhibited by hexobarbitone (van Zwieten, 1969). The influence on calcium metabolism has been studied only in brain slices, where Klaus (1967) demonstrated a significant inhibition of the Ca^{2+}-efflux by sodium hexobarbitone. As no report of the influence of barbiturates on cellular calcium metabolism in intact heart muscle tissue appears to have been published, we have examined the influence of sodium hexobarbitone on calcium ion movements in intact atria of the guinea-pig. The effect of the drug was investigated both in beating atria and in resting left auricles.

METHODS

Isolated atria or left auricles were dissected from guinea-pigs of either sex, 350–450 g, according to Hoditz & Lüllmann (1964). The left auricles, being devoid of pacemaker cells did not beat spontaneously. The organs were suspended in Muralt Tyrode solution that was continuously gassed with 5% carbon dioxide in oxygen. The calcium ion content of the medium was 1·8 mmol/litre. The temperature was maintained at 30° throughout. Supramaximal stimulation with rectangular pulses (duration 2 ms) at a frequency of 180/min was with a stimulator from Braun GmbH, Melsungen (W.-Germany). The organs were attached to a strain gauge, connected to an amplifier and Helcoscriptor recorder (type HE 86 t). The volume of the bath was 20 ml. The experiments were limited to a barbiturate concentration of $7{\cdot}8 \times 10^{-4}$ M, since at this concentration the contractile force was reduced by half of its initial value.

Calcium metabolism

Before addition of drug to the medium the organs were equilibrated in Tyrode solution at 30° for 30 min. During equilibration of the isolated atria electrical stimulation was applied. In all experiments, to allow a steady-state reduction of beat in presence of hexobarbitone to be reached, the drug was added to the medium before the uptake of ^{45}Ca was measured. Where the uptake of $^{45}Ca^{2+}$ was studied, the bath

volume was 750 ml. The uptake of $^{45}Ca^{2+}$ by the isolated atria or left auricles was determined according to Hoditz & Lüllmann (1964), Lahrtz, Lüllmann & van Zwieten (1967) and Haacke, Lüllmann & van Zwieten (1970). The total calcium content of the organs was determined spectrofluorimetrically (Zepf, 1966). Sodium hexobarbitone was obtained from Bayer AG, Leverkusen. $^{45}CaCl_2$ (specific activity of the stock solution 5 Ci/mmol) was supplied by the Radiochemical Centre, Amersham, U.K.

RESULTS AND DISCUSSION

Sodium hexobarbitone ($7{\cdot}8 \times 10^{-4}$ M) diminished the contractile force of electrically stimulated atria by 58 ± 4% of the initial value (mean ± s.e., n = 6). The initial steep phase of the uptake curves (Fig. 1) probably represents the exchange of Ca^{2+} in the extracellular space against $^{45}Ca^{2+}$ from the medium (Lahrtz & others, 1967). From Fig. 1 it is obvious that the rate of uptake of $^{45}Ca^{2+}$ by beating atria was reduced in the presence of sodium hexobarbitone ($7{\cdot}8 \times 10^{-4}$M). Both in control experiments and in the presence of the drug, equilibrium was achieved after approximately 180 min of incubation. The equilibrium uptake (at t = 180 min) was not affected by the presence of hexobarbitone. Both in control experiments and also in presence of the

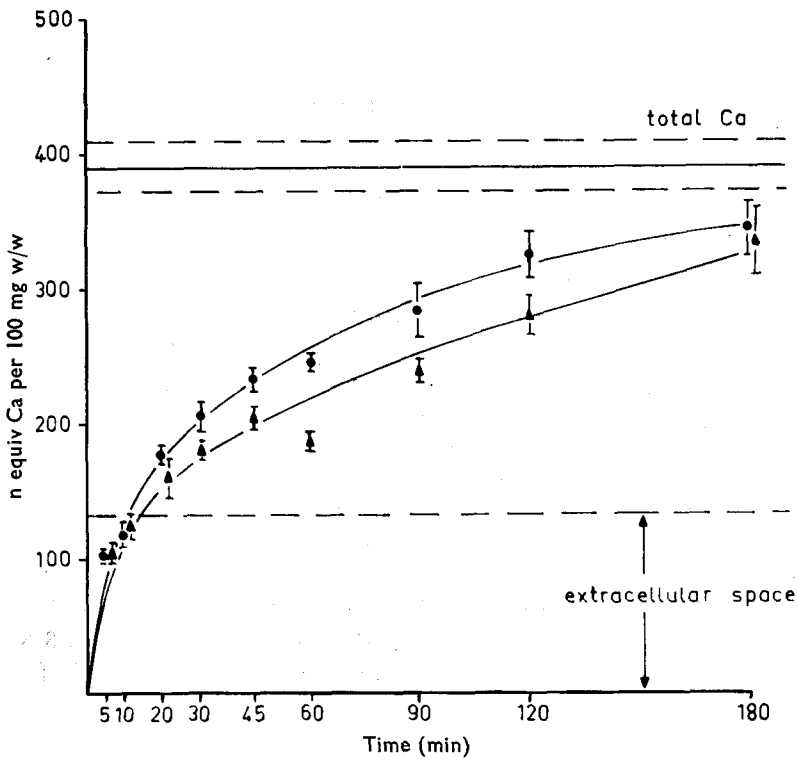

FIG. 1. Uptake of ^{45}Ca by electrically stimulated atria. Frequency of beating 180/min. Calcium content of the Tyrode solution 1·8 mmol/litre. Ordinate: n equiv calcium taken up from the ^{45}Ca-containing medium. The amount of calcium taken up was calculated from the tissue radioactivity. Each point on the curves represents the mean value (±s.e.) for 8–10 different organs. The size of the extracellular space is also shown (dotted line). The amount of calcium present in the extracellular compartment was calculated, the extracellular space amounting to 0·3 ml/g tissue. ○ - - - ○ ^{45}Ca uptake under control circumstances. △ - - - △ ^{45}Ca uptake in the presence of sodium hexobarbitone ($7{\cdot}8 \times 10^{-4}$ M).

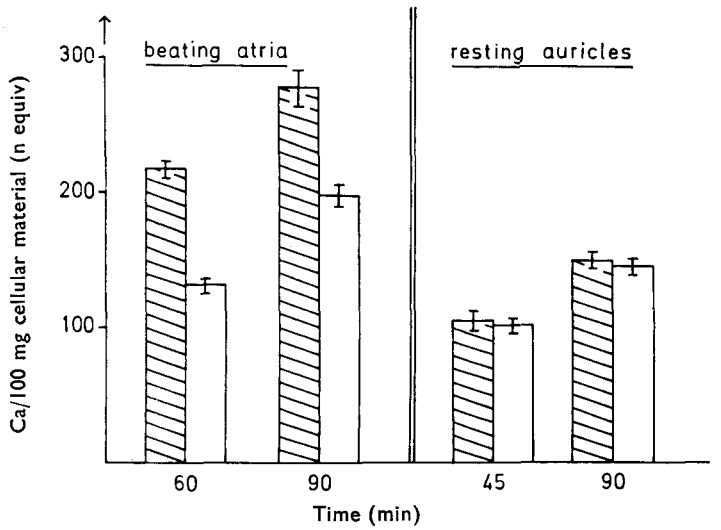

FIG. 2. Cellular exchange of calcium under influence of sodium hexobarbitone, determined by means of ^{45}Ca. For the calculations of the exchange the extracellular space of atrial tissue was assumed to be 0·3 ml per g tissue. The columns represent the mean values (± s.e.) for 8–10 different organs. Hatched columns: controls; open columns: sodium hexobarbitone ($7{\cdot}8 \times 10^{-4}$M).

barbiturate the total calcium concentration of the isolated organs remained unchanged throughout the experiments (control experiments: 383 ± 14 n equiv/100 mg, n = 71; hexobarbitone experiments: 395 ± 21 nl equiv/100 mg, n = 68).

To assess drug effects on calcium exchange, the cellular uptake of $^{45}Ca^{2+}$ was calculated in terms of nequiv Ca/100 mg cellular material. For these calculations an extracellular space of 0·3 ml/g tissue was assumed on the basis of previous measurements of [^{14}C]saccharose (Bauer, Lüllmann & Richter, 1963; Lüllmann & van Zwieten, 1967). Checking experiments using [^{14}C]saccharose confirmed this value and showed that electrical stimulation and the addition of hexobarbitone did not modify the saccharose space.

The results of the calculations are shown in Fig. 2. The differences in ^{45}Ca uptake owing to drug treatment already observed in Fig. 1 were also obvious if the amount of cellular calcium exchanged was taken into account. The determination of the ^{45}Ca-concentration and the total calcium content of the organs allows the calculation of the so-called exchangeable calcium fraction, which is obtained from the relation:

$$\text{exchangeable fraction} = \frac{\text{specific activity of Ca in the tissue}}{\text{specific activity of Ca in the medium}}$$

Since in our studies the total calcium content of the tissues remained virtually unchanged, a certain parallelism will exist between the drug-induced changes in ^{45}Ca uptake and those in the calculated exchangeable fraction (see Table 1). As shown in Fig. 2, the presence of $7{\cdot}8 \times 10^{-4}$M sodium hexobarbitone did not influence the uptake of ^{45}Ca by *resting* left auricles. In comparison with beating atria the rate of uptake of ^{45}Ca by resting organs under control circumstances was much lower (Fig. 2), agreeing with Hoditz & Lüllmann (1964). Both under control circumstances and in a hexobarbitone-containing medium the total calcium content of resting auricles remained unchanged throughout the experiments (controls: 377 ± 14 n equiv/100 mg wet weight, n = 25; hexobarbitone: 391 ± 19 n equiv/100 mg wet weight, n = 22).

Table 1. *Exchangeable calcium fraction in beating atria and in resting auricles.* Influence of sodium hexobarbitone ($7{\cdot}8 \times 10^{-4}$M). The exchangeable fraction was calculated from the data in Figs 1 and 2 (details see text) and was expressed as percentage of total tissue Ca.

	Beating atria exchangeable fraction (%)		Resting auricles exchangeable fraction (%)	
	60 min	90 min	45 min	90 min
Controls	50	69	20	40
Sodium hexobarbitone ($7{\cdot}8 \times 10^{-4}$M)	34	54	20·5	40

In contrast to beating organs the exchange in *resting* left auricles was not affected by sodium hexobarbitone. This observation would suggest that only the additionally occurring membrane permeability for Ca^{2+} during excitation is impaired by sodium hexobarbitone whereas *at rest* the barbiturate does not interfere with the passive calcium movements. Similar observations have been made for K^+ in atrial-tissue (Klaus & Lüllmann, 1971) and for Ca^{2+} and K^+ in brain slices that were studied at rest or when exposed to electrical stimulation (Klaus, 1967).

According to Chimoskey & Gergely (1968), differences in pH of the medium might account for the changes in calcium movements observed in isolated sarcoplasmic reticulum preparations that were exposed to barbiturates (Briggs, Gertz & Hess, 1966). But the pH of the media we used was in the range 7·2–7·3. However, it could be argued that the intracellular pH might be modified and could be of greater significance in affecting cellular calcium disposition than alterations of extracellular pH (cf. Waddell & Bates, 1969).

The present experiments indicate that the amount of calcium entering the cell per single excitation will be reduced in the presence of hexobarbitone. However, it might also be that the number of calcium ions mobilized within the cell is diminished in the presence of hexobarbitone as methods and calculations do not allow any differentiation between these two processes.

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