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Magnesium fluxes in ventricular fibrillation and defibrillation in untreated and dibenzepine HC1$^{(R)}$ pretreated cats

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Summary: Serum magnesium concentration (S-Mg) was estimated in 12 anesthetized cats before and after central thoracotomy, during an electrically induced ventricular fibrillation (VF) and after defibrillation (DEF), and again, in the same experimental animals, after the administration of 3mg/kg of dibenzepine HC1$^{(R)}$ – a tricyclic antidepressant reported to facilitate spontaneous DEF[1] – as well as during a subsequently induced VF and after the spontaneous DEF which followed. In the first part of the experiment, the surgery and the induction of VF caused no significant change of mean serum magnesium concentration (S-Mg) or serum calcium concentration (S-Ca), whereas the DEF was accompanied by Mg efflux (a significant increase of mean S-Mg from 0.824 mmol/l, SD 0.182, $n=12$ to 0.991 mmol/l, SD 0.182, $n=12$; $P<0.05$). In the second part of the experiment, following the administration of dibenzepine HCl$^{(R)}$ there was Mg influx (a lowering of mean S-Mg to 0.891 mmol/l, SD 0.160, $n=12$; $P<0.05$). During VF in the pretreated cats, S-Mg remained unchanged, while S-Ca decreased significantly ($P<0.05$), followed by a rebound Ca^{++} efflux (a systematic rise of S-Ca) as the animals defibrilated spontaneously. Concomitantly, there was Mg efflux (a systematic rise of S-MG), the mean S-Mg rising from 0.879 mmol/l, SD 0.143, $n=9$ to 1.083 mmol/l, SD 0.257, $n=7$, ie to virtually the same value as that obtained after electrically induced DEF. Conclusion: (1.) Whether electrically or dibenzepine HCl$^{(R)}$ induced DEF is accompanied by Mg^{++} efflux, suggesting that in both cases, the antiarrythmic action is mediated by a rise of extracellular Mg^{++}. (2.) Administration of dibenzepine HCl$^{(R)}$ appears to cause Mg influx, encourages Ca influx during VF, but during the following spontaneous DEF, Ca efflux is concomitant with Mg efflux.

Key Word: Magnesium, calcium, ventricular fibrillation, defibrillation, dibenzepine HCl$^{(R)}$.

Introduction

Optimal concentration of potassium and magnesium are required for normal function of the cardiac muscle and the administration of magnesium has been shown to arrest refractory cardiac arrythmias[2,3] diminish the number of ventricular extrasytoles in man[4] and to cause immediate disappearance of torsade de pointes[5]. In experimental animals magnesium was shown to reduce the threshold for the stimuli inducing defibrillation[6], possibly by terminating triggered activity and supressing transient depolarizations[7].

Since dibenzepine (HCl$^{(R)}$) ('Noveril', Wonder), a tricyclic antidepressant, was reported to facilitate spontaneous defibrillation[1], it was of interest to investigate possible underlying changes in serum magnesium (S-Mg) and serum calcium concentration

(S-CA) during ventricular (VF) and defibrillation (DEF) in untreated, as well as in dibenzepine HCl(R) pretreated animals.

Methods

Reference values

In order to establish the reference values for divalent cations in the cat, S-Mg was estimated in 20 cats (7 females and 11 males; mean weight 3.28 kg, SD 0.5) and the mean S-Ca in 13 cats (4 females and 9 males; mean weight 3.0 kg, SD 0.57).

The experimental design

Following an electrically induced VF, spontaneous defibrillation is found to occur in about 10 per cent of cats, without any pharmacological intervention. Consequently, before the administration of dibenzepine HCl(R), an electrically induced VF had to be applied first in the untreated state (see 'First part of the experiment'), in order to exclude spontaneously defibrillating cats. Experimental animals which did not defibrillate spontaneously, were electrically defibrillated and on normalization of the physiological parameters (blood pressure and ECG tracing) entered into the experiment (see 'Second part of the experiment'). The advantage of this design was that each animal could serve as its own control (see 'Statistics').

Initial experimental procedure

Twelve cats (4 females and 8 males; mean weight 3.18 kg, S.D. 0.5) anaesthetized using sodium pentobarbital (25–30 mg/kg body weight), their femoral arterial pressure measured with the aid of a Statham pressure transducer and recorded simultaneously with ECG limb leads, using a 'Grass' polygraph, were tracheotomized and respirated with a Harvard respirator, using room air, and their femoral arterial blood was collected for biochemical screening to establish the baseline values (see 'Laboratory estimations').

First part of the experiment

Central thoracotomy was performed to expose the heart, which was cradled in the open pericardium (step I). When the blood pressure (BP) and ECG tracing normalized, VF was induced, using a 'Grass SD 9' stimulator. The fibrillating stimuli consisted of a gated train of rectangular pulses (frequency of 100 pulses/s′, strength 10–15 V and duration of 0.5–1.0 ms′), delivered through two silver needle electrodes attached to the left ventricular epicardium. The VF was induced for < 2 min ($\bar{x}$ mean VF time 149.2, SD 54.45′, $n = 6$) (Step II), and the DEF was carried out using 'Electrodyne D-33' defibrillator (50–100 V; Step III; Fig. 1). The experimental animals were now rested until the physiological parameters (BP, ECG) normalized before they entered the second part of the experiment.

Second part of the experiment

In the second part of the experiment, dibenzepine HCl(R) was injected i.v. (*3 mg/kg body weight*) (Step IV). Ten minutes later, a second VF was electrically induced (Step V). This time the animals defibrillated spontaneously and the physiological parameters normalized quickly (Step VI; Fig. 2).

Laboratory estimations

Blood was collected at each step of the experiment, through an indwelling catheter, from the femoral artery (we found that is satisfactorily reflected the intracardiac fluxes, as found on comparing few

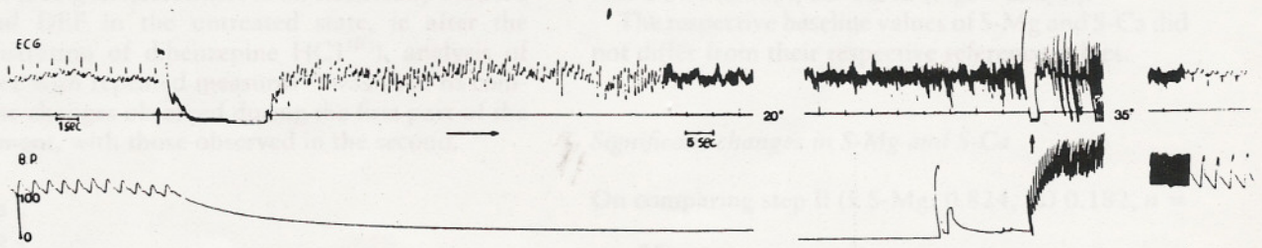

Fig. 1. Untreated cat. = electrically induced VF the duration of VF was 125″. The VF was terminated by electrical defibrillation. The normalization of BP and ECG, after cessation of VF, occurred after 45″.

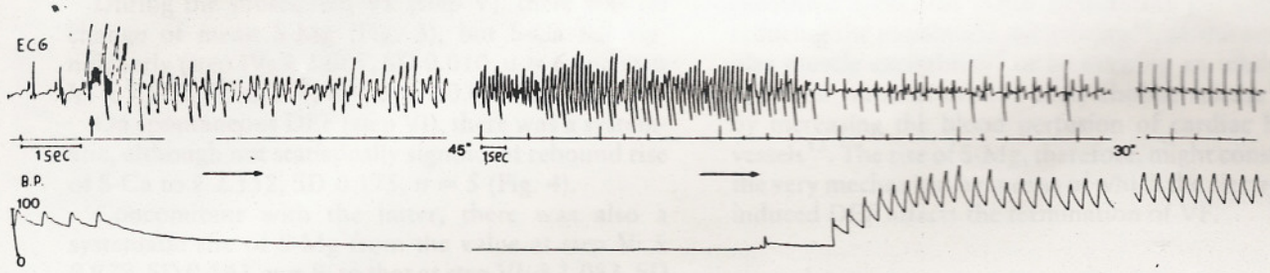

Fig. 2. Dibenzepine HCL(R) pretreated cat. = electrically induced VF. The duration of VF was 65". The normalization of BP and ECG, after the cessation of VF, ocurred after 43".

randomly chosen sample estimates of blood drawn from the heart with those obtained from the femoral artery). S-Ca, creatinine total protein, albumin and globulin were estimated in an autoanalyzer, and S-Mg, in a Perkin Elmer No. 305 A, atomic absorption spectrophotometer, using Sr_2Cl as a diluent[8].

The VF and DEF appeared to favour occasional occurrence of haemolysis and/or a tendency to increased coagulability of the collected blood. Only faultless samples were monitored and worked through in the statistics; experimental animals which showed either of the above phenomena in more than two samples were excluded from the experiment.

Statistics

Paired Student's *t*-test was used for comparison of the laboratory values between each two steps, and with baseline and reference values. Since in the experimental design used, each animal served as its own control (being subjected first to an electrically induced VF and DEF in the untreated state, ie after the administration of dibenzepine HC1(R)), analysis of variance with repeated measures[16] was used to compare the changes observed during the first part of the experiment, with those observed in the second.

Results

Duration of spontaneous defibrillation

Following dibenzepine HC1(R) administration, all the animals defibrillated spontaneously; mean VF time: 44.8$^{3'}$ (SD 41.0, n = 7).

Laboratory reference values

The reference value for S-Mg in the cat was: $\bar{x}$ 0.948, SD 0.272 mmol/l, n = 20. The reference value for S-Ca in the cat was: $\bar{x}$ 2.385 mmol/l, SD 0.224, n = 13.

Baseline values

The mean baseline S-Mg of the examined cats was 0.904 mmol/l, SD 0.309, n = 12, and the mean baseline S-Ca (estimated in 9 cats; 7 males, 2 females) was 2.394 mmol/l, SD 0.202 (Figs 3 and 4.).

The respective baseline values of S-Mg and S-Ca did not differ from their respective reference values.

Significant changes in S-Mg and S-Ca

On comparing step II ($\bar{x}$ S-Mg; 0.824, SD 0.182, n =

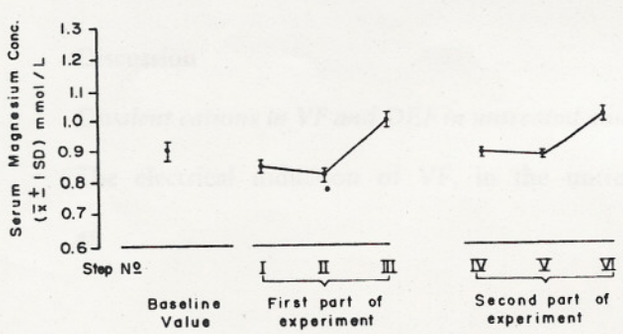

Fig. 3. S-Mg concentration (X ± 1SD) at baseline value and each step of the experiment.

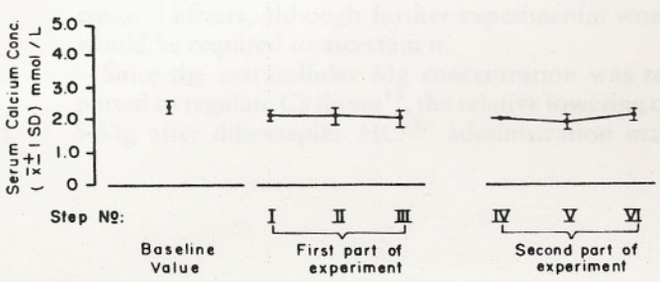

Fig. 4. S-Ca concentration ($\bar{X}$ ± 1SD) at baseline and at each step of the experiment.

12) with step III ($\bar{x}$ S-Mg; 0.991, SD 0.209, $n = 12$) there was a significant rise of mean S-Mg ($P<0.05$; Fig 3).

At step IV following administration of dibenzepine HC1$^{(R)}$ ($\bar{x}$ S-Mg; 0.891, SD 0.160, $n = 12$) there was a significant decrease of S-Mg ($P<0.05$) in relation to the preceding step, but not in relation to the baseline value (Fig. 3).

During the subsequent VF (step V), there was no change of mean S-Mg (Fig. 3), but S-Ca fell significantly (step IV: $\bar{x}$ 2.087, SD 0.010, $n = 6$ and step V: $\bar{x}$ 1.868, SD 0.214, $n = 6$; $P<0.05$; Fig. 4).

On spontaneous DEF (step VI), there was a systematic, although not statistically significant rebound rise of S-Ca to $\bar{x}$ 2.152, SD 0.175, $n = 5$ (Fig. 4).

Concomitant with the latter, there was also a systematic rise of S-Mg from the value at step V: $\bar{x}$ 0.879, SD 0.143, $n = 9$, to that at step VI: $\bar{x}$ 1.083, SD 0.257, $n = 7$, the latter value being virtually the same as that obtained after electrically induced DEF (step III; Fig. 3).

On analysis of variance with repeated measures, used in order to compare the dynamic changes in the first part of the experiment (steps I–III) with those of the second part of the experiment (steps IV–VI), there was no significant change in the pattern of S-Mg fluxes in both parts of the experiment (Fig. 3). As regards S-Ca, we found a significant interaction, ie that the trend of S-Ca changes between the steps of the first and those of the second part of the experiment differed significantly ($F = 7.39$, df 2, 6, $P<0.05$) as seen in Fig. 4.

Other laboratory values

There were no statistically significant differences in the remaining laboratory variables estimated, both on comparison between reference and baseline values and consecutive steps (paired Student's *t*-test).

On analysis of variance with repeated measures, total protein and globulin differed significantly between the two parts of the experiment ($F = 52.2$, df 1, 3, $P<0.01$, and $F = 17.2$, df 1, 3, $P<0.05$, respectively), showing lower values in its second part, however, on inspecting the means, one sees that it is rather a gradual fall of total protein and globulin levels throughout the whole experiment. Serum albumin values, after a non-significant initial fall, following tracheotomy (step I), remained virtually unchanged throughout.

Discussion

Divalent cations in VF and DEF in untreated animals

The electrical induction of VF, in the untreated animals, did not cause any significant change of the divalent cation concentration, but the electrically induced DEF did cause a significant rise of S-Mg to a mean of 0.991 mmol/l, and no change of S-Ca.

Magnesium has been reported to induce, or facilitate, defibrillation[6,9,10]. Therefore, the rise of extracellular Mg, which accompanied the DEF, might constitute a decisive factor in inducing the DEF, by reducing the membrane excitability[11], or the ventricular muscle excitability[7] or by exciting an inhibitory effect on stimulus evoked acetylcholine release[12], or by increasing the blood perfusion of cardiac blood vessels[13]. The rise of S-Mg, therefore, might constitute the very mechanism by means of which the electrically induced DEF affects the termination of VF.

Divalent cations in dibenzepine HCL$^{(R)}$ pretreated animals

Tricyclic antidepressants in high dosage are known to give rise to cardiac toxicity and arrhythmias[14]. However, in the doses used (3mg/kg), which are within the pharmological range, dibenzepine HCl$^{(R)}$ ('Noveril', Wander), a tricyclic antidepressant, was shown to cause spontaneous termination of electrically induced VF. This has been confirmed by our study; we found spontaneous DEF in all the experimental animals, after pretreatment with dibenzepine HCl$^{(R)}$.

The administration of dibenzepine HCl$^{(R)}$ caused a significant lowering of mean S-Mg in comparison with that of the preceding step, but not in relation to the baseline value. In order to establish with more certainty whether it was the administration of the drug itself which caused the lowering of S-Mg, or, whether the latter merely represented a further return to a steady state after the conclusion of the first part of the experiment, we looked at two additional cats in which we did not induce VF, as a preliminary, but merely allowed ½ h. for recovery after the thoracotomy before administering dibenzepine HCl$^{(R)}$, in order to follow the dibenzepine HCl$^{(R)}$ effect alone, both immediately after its administration, as well as at 10 and 30 minutes later. In both cats, we noticed a downward trend of S-Mg, which amounted to an average fall of 14 per cent immediately after the administration, of the drug, a decrease by 24 per cent after 10 minutes, and by 30 per cent, after 30 minutes. This strongly suggests that the lowering of S-Mg seen after dibenzepine HCl$^{(R)}$ administration may be due to the latter and indeed constitute one of its pharmacologial effects, although further experimental work would be required to ascertain it.

Since the extracellular Mg concentration was reported to regulate Ca fluxes[13], the relative lowering of S-Mg after dibenzepine HCl$^{(R)}$ administration may

have accounted for the Ca influx during the subsequent VF – a phenomenon not seen in the untreated animals, in which the induction of VF did not cause any signifiant change in S-Mg. Alternatively, it is possible that dibenzepine HCl(R) may have facilitated the opening of the voltage dependant CA^{++} channels in response to the electrical pulses used to induce the VF, whereas, the latter had no effect on Ca channels in the untreated animals.

Magnesium may either occupy the non-specific Ca^{++}-Mg^{++} binding sites on the regulatory muscle contractile proteins, thus competing with Ca^{++}, or it may shield the Ca^{++} specific binding sites of the regulatory contractile proteins from rapid Ca^{++} transients.[15] Therefore, if we assume that dibenzepine HCl(R) does indeed cause Mg influx, the latter mechanism could explain the rapid rebound Ca efflux which took place simultaneously with the spontaneous DEF.

Simultaneously with the spontaneous DEF we saw a systematic rise of S-Mg, its mean rising to 1.083 mmol/l ie virtually to the same value, as that resulting from the rise of S-Mg during the electrically induced DEF.

Conclusion

Our findings suggest that the electrically induced DEF as well as the spontaneous DEF, caused by pretreatment with dibenzepine HCl(R), are both mediated through a rise of extracellular magnesium, which, as reported by several workers, has an antiarrythmic action.

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