Protection of Chlorpromazine-Induced Arrhythmia by Flavin-Adenine-Dinucleotide in Canine Heart

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Summary

To investigate the mechanism of chlorpromazine (CPZ)-induced ventricular arrhythmia, the changes in ventricular fibrillation threshold (VFT) were followed after intravenous injection of CPZ (1 mg/Kg) in dogs. Following injection, VFT was decreased to 56.6±5.4% (mean ±SE) of the initial level. Since flavin-adenine-dinucleotide (FAD) combines specifically with CPZ in vitro, we investigate whether or not prior treatment with FAD prevents the CPZ effect. With FAD (2 mg/Kg), the CPZ-induced decrease in VFT was significantly cancelled (92.2±4.2% of the initial level). Mitochondria isolated from canine heart after CPZ injection showed a significant decrease in respiratory control index and ADP/O. Effects of CPZ on canine heart mitochondria were also well cancelled by prior administration of FAD. The findings suggest that the arrhythmogenic action of CPZ might be associated in part with impaired function of heart mitochondria. These results also suggest that FAD might be useful in the treatment of the cardiac disturbances associated with overdosage of CPZ.

Additional Indexing Words:
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It is often observed that ventricular arrhythmia or sudden death occurs in patients treated with psychotropic drugs, such as chlorpromazine (CPZ).1-8 These complications have been encountered in relatively young patients, so it is generally considered that such phenomena are attributed not to arteriosclerotic coronary artery disease, but to direct cardiotoxicity of the psychotropic drugs. Since specific antidote against CPZ has not been elucidated, the treatment of these adverse reactions is only symptomatic.

We have previously demonstrated in vitro that flavin-adenine-dinucleotide (FAD) combined specifically with CPZ.9 In the present study, we investigate effects of CPZ on ventricular fibrillation threshold (VFT) and on blood pressure. It is also intended to determine whether or not FAD pro-
ects against these effects of CPZ. CPZ acts directly on several enzyme systems in mitochondria. According to some investigators, the cardiac effects of CPZ might be mediated through its effects on mitochondrial functions. We also investigate the effects of CPZ on canine heart mitochondrial function and the protection afforded by FAD against these effects.

Materials and Methods

Eighteen adult mongrel dogs weighing 8 to 12 Kg were used. The dogs were anesthetized with intraperitoneal pentobarbital sodium (50 mg/Kg), and placed under artificial respiration. Then they were thoracotomized along the 5th intercostal space to expose the heart, and a platinum stimulating electrode with an interpolar distance of 3 mm was attached to the apex of left ventricle.

The measurement of VFT was described in detail previously. Briefly, a series of rectangular pulses (100 Hz, 1 msec duration) were delivered through the electrode attached to left ventricle to cover fully the ventricular vulnerable period. The intensity of pulses was increased gradually until more than 5 extrasystoles occurred successively to obtain VFT. The aortic blood pressure was measured through a catheter cannulated into the carotid artery and connected to an manometer (Nihon Koden Ltd, MP-4T). Catheters were also inserted into both femoral veins, one was used for injection of the drugs and the other for withdrawing blood samples. Throughout the experiment, a lead II ECG was monitored.

The dogs were divided into 3 groups and they were received the following treatments. Each group consisted of 6 dogs. Dogs in group I as control, were given 2 ml/Kg of physiological saline by intravenous injection. Ten min after the start of the first injection, another dose of saline, 1 ml/Kg, was injected intravenously. Dogs in group II were given i.v. 2 ml saline/Kg. Ten min afterwards, 1 mg CPZ/Kg was injected. Dogs in group III were given i.v. FAD, 2 mg/Kg. Ten min later, they were given CPZ, 1 mg/Kg. All solutions was administrated in 1 min or 2. Blood samples were taken before and 10, 20, 30, and 40 min after the intravenous injection of saline (groups I and II) or FAD (group III). Serum K⁺ and blood pH were also measured. Heart rate, blood pressure and VFT of each dog were recorded at the same intervals. Forty min after the first i.v. injection, the heart was isolated from each dog. Myocardial mitochondria were prepared by the method of Hatefi et al. In order to measure the respiratory control index (RCI) and ADP/O of the heart mitochondria, 2.8 ml of mannitol reaction mixture (0.3 M mannitol, 10 mM phosphate, 2.5 mM MgCl₂, 10 mM KCl, and 0.25 mM EDTA, pH 7.4) and 0.3 ml of the mitochondrial sample (10 mg mitochondrial protein per ml) were added, together with 0.1 ml of potassium succinate (0.2 M) and 0.05 ml of ADP (0.01 M) as substrates, to a cell of the closed assay system. Respiration was followed by means of an oxygen electrode (Kyusui Kagaku, Ltd). To assess the effects of CPZ and FAD on mitochondrial functions in vitro, using heart mitochondria prepared from the intact dogs, RCI and ADP/O were determined under 3 different conditions, viz. (1) without addition of drug, (2) with addition of CPZ (50 µg), (3) with additions of FAD (100 µg) and CPZ (50 µg) to the mannitol reaction mixture in the cell of the closed assay system.
Fig. 1. Plots of percent changes in VFT (ordinate) against time (abscissa) in groups I, II, and III. VFT is significantly decreased after injection of chlorpromazine, however, FAD prevents significantly the effect of chlorpromazine. S=saline; F=FAD; C=chlorpromazine. Statistical analysis is determined by Student’s t-test.

Fig. 2. Plots of changes in systolic (top) and diastolic (bottom) blood pressures (ordinate) against time (abscissa) in groups I, II, and III. S=saline; F=FAD; C=chlorpromazine. In group II, blood pressure, both systolic, and diastolic, is decreased significantly, however in group III the effect of chlorpromazine is fairly well suppressed.
RESULTS

In Fig. 1, VFT changes observed in the 3 groups are shown. No significant changes in VFT occurred in group I, while group II showed a significant decline in VFT (1.11±0.15 mA, mean±SE) to 56.6±5.4% of the initial level 20 min after injection of CPZ, that is, 30 min after the start of the experiment. In group III, which had received FAD before CPZ, a decrease in VFT (1.78±0.14 mA, i.e., 92.2±4.2% of the initial level) was observed. However, it was significantly protected comparing with group II. Systolic and diastolic blood pressure changes observed in those 3 groups are shown in Fig. 2. Group II exhibited a decrease in both systolic and diastolic pressures by 25.0±3.9 mmHg and 23.0±4.0 mmHg, respectively, 40 min after the start of the experiment comparing with the initial level. Drops in blood pressure in group III were only 8.0±4.2 mmHg (systole) and 8.3±3.8 mmHg (diastole) 40 min after the start of the experiment. The dogs showed a transient hypotension within 10 min after injection of FAD, then their blood pressures recovered to the initial level. The blood pH, serum K+ and heart rate at various times following the initiation of the experiment showed no significant changes.

Fig. 3. Respiratory control index (RCI) and ADP/O of mitochondria from groups I, II, and III. In group II, significant decreases of RCI and ADP/O are observed. FAD also prevents mitochondrial dysfunction induced by chlorpromazine.
The RCI and ADP/O which reflect the mitochondrial functions of myocardial cells are presented in Fig. 3. Compared with the values of $4.2 \pm 0.8$ for RCI and $1.94 \pm 0.02$ for ADP/O in group I, group II showed significantly lowered values for these parameters, $2.7 \pm 0.05$ and $1.53 \pm 0.07$, respectively. Group III also showed significantly lowered values of $3.6 \pm 0.04$ and $1.72 \pm 0.03$, but the declines in mitochondrial functions were not as remarkable as those in group II. Representative traces of mitochondrial respiration in the 3 groups are presented in Fig. 4. The mitochondrial respiratory pattern of group II was characterized by enhanced State IV respiration and an indistinct shift to "re-State IV" respiration. (After adding of ADP, the mitochondrial respiration rate shifts from State IV to State III, and, after phosphorylation of the added ADP is completed, it returns from State III to re-State IV). In group I and also in group III, this re-shift was more clear than that of group II. Such a phenomenon was observed in the in vitro experiment as well. The mitochondrial respiratory curve obtained with a reaction mixture after adding 50 µg of CPZ showed a RCI of $3.1 \pm 0.05$ and an ADP/O of $1.60 \pm 0.04$, whereas with a reaction mixture contain-

![Fig. 4](image-url)
Fig. 5. Respiratory control index (RCI) and ADP/O of mitochondria in vitro with additions of FAD and subsequently chlorpromazine (FAD), those with only chlorpromazine (CPZ) and those without addition of FAD or chlorpromazine (Cont.). With chlorpromazine, RCI, and ADP/O are significantly decreased. FAD prevents the effects of chlorpromazine.

ing 100 μg of FAD and 50 μg of CPZ, the RCI was 3.7 ± 0.05 and the ADP/O 1.83 ± 0.03. The control group showed corresponding values of 4.6 ± 0.10 and 2.00 ± 0.00 (Fig. 5).

DISCUSSION

In this series of experiments, we observed that CPZ had arrhythmogenic effect and hypotensive action and that FAD effectively cancelled out these CPZ effects (Figs. 1 and 2). Various ECG abnormalities induced by CPZ, such as tachycardia, ventricular arrhythmia, abnormal T waves, and prolongation of QT interval have been reported.1)-8) Ventricular arrhythmia is particularly of clinical importance as it may cause sudden death. Arita et al12) postulated that ventricular arrhythmia during CPZ therapy resulted from a prolongation of the ventricular vulnerable period. Some mechanisms are considered to explain the hypotensive effect of CPZ; 1. antagonism against adrenalin and noradrenalin,13) 2. diminution of peripheral vascular resistance,14),15) 3. suppression of myocardial activity,16) 4. effect via the central nervous system.17) However, the precise mechanism of the arrhythmogenic
as well as hypotensive effects of CPZ remains unclear. We have previously reported\(^{18}\) that, after the injection of CPZ to patients, irregular slow waves of high voltage appeared in the electroencephalogram (EEG) and that the administration of FAD partially reversed the effect of CPZ on EEG. Similarly, in the present study, the effect of CPZ on canine heart is cancelled by FAD. We have demonstrated \textit{in vitro} the formation of CPZ-FAD complex and competition of these compounds for the apoenzymes of flavin-containing enzyme.\(^{9}\) On the other hand, CPZ inhibits not only flavin-containing enzymes, but also hexokinase, tricarboxylic acid cycle enzymes, cytochromes,\(^{19}\) and oxidative phosphorylation.\(^{20}\) This may be the reason why FAD can not reverse the CPZ effect completely. However, FAD did protect well against the mitochondrial dysfunction induced by CPZ (Figs. 4 and 5). Since mitochondria contain both flavin-containing enzymes and others, it is possible that FAD cancels the CPZ effect competitively, because the structures of these 2 substances are essentially similar (Fig. 6).

In this report, to investigate the biochemical effects of CPZ, we examined mitochondrial function, because 1. mitochondria contained oxidative phosphorylation system including cytochromes which were inhibited by CPZ, 2. it was rather easy to prepare mitochondria and to measure their degree of dysfunction quantitatively, 3. there was a study by Alexander and Nino,\(^5\) reporting that both ECG abnormality and morphological changes of mitochondria were observed in patients treated with CPZ. We also observed that the administration of CPZ lowered VFT and blood pressure, and caused mitochondrial dysfunction simultaneously. FAD prevented these CPZ effects. There was no significant difference in serum K\(^+\), blood pH, and heart

\[\text{Chlorpromazine} \quad \text{FAD}\]

\(\text{Fig. 6. Chemical structures of chlorpromazine and FAD.}\)
rate among the groups. It supports that arrhythmogenic effect of CPZ has some relationships with mitochondrial dysfunction. It is well known that mitochondria produce the majority of the ATP, which is required for all energy demanding processes in a living body. For example, using ATP, Na\(^+\)-K\(^+\) ATPase exchanges Na\(^+\) and K\(^+\) across the cell membrane. In addition, we have reported\(^{21}\) that elevated serum FFA by injection of lipid emulsion induced ventricular arrhythmia and simultaneously mitochondrial dysfunction, so some relationships might exist between arrhythmogenicity and mitochondrial function. Also, we would like to point out that FAD might be useful in the treatment of the cardiac disturbances associated with overdosage of CPZ.

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References


