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# The effects of amiodarone on the electrocardiogram of the guinea-pig are not explained by interaction with thyroid hormone metabolism alone

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**1** The iodine-containing contrast medium iopanoic acid induces alterations of thyroid hormone metabolism comparable to those observed with the iodine-containing antiarrhythmic drug amiodarone. Both compounds inhibit the intracellular conversion of thyroxine ($T_4$) to triiodothyronine ($T_3$). Using iopanoic acid, the question was investigated, in guinea-pigs, whether inhibition of $T_4 \rightarrow T_3$ conversion is by itself associated with the same changes in the electrocardiogram, i.e. QT prolongation and bradycardia, as those observed during amiodarone treatment.

**2** At a dose of $4\,g\,kg^{-1}$, iopanoic acid induced maximal inhibition of the $T_4 \rightarrow T_3$ conversion. Although these changes were even more pronounced than those in a control group of animals treated with $2.12\,g$ amiodarone $kg^{-1}$, neither prolongation of the QT nor a slowing of the heart rate was observed. QT prolongation and bradycardia were induced only by amiodarone treatment but not by iopanoic acid.

**3** Iopanoic acid at the high toxic dose of $12\,g\,kg^{-1}$ induced the same degree of inhibition of $T_4 \rightarrow T_3$ conversion as the $4\,g\,kg^{-1}$ dose. QT prolongation and slowing of the heart rate were apparent at this dose in parallel with a loss of weight.

**4** It is concluded that even a maximal inhibition of the $T_4 \rightarrow T_3$ conversion has no effect on the ECG of guinea-pigs. The inhibition of the $T_4 \rightarrow T_3$ conversion alone does not explain the QT prolongation and bradycardia observed with amiodarone treatment. The amiodarone effects on the ECG may represent a combination of interactions with thyroid hormones and antiadrenergic activity.

## Introduction

Although there are many accounts of the clinical use of the potent antiarrhythmic drug amiodarone (Rosenbaum *et al.*, 1976; Coumel & Fidelle, 1980; Garson *et al.*, 1984), there are still very few studies on the mechanisms of action of this extraordinary drug. This is surprising because it has long been known that the non-competitive α- and β-adrenoceptor blocking actions of amiodarone (Charlier *et al.*, 1967) do not fully explain its various cardiac effects.

Singh & Vaughan Williams (1970) recognized for the first time that the electrocardiographic alterations observed in amiodarone-treated animals were indistinguishable from those present in hypothyroid animals (Freedberg *et al.*, 1970). They therefore concluded that amiodarone might interfere with the effects of thyroid hormones on the heart (Singh & Vaughan Williams, 1970). The important observation by Burger *et al.* (1976), that amiodarone inhibits the 5′ monodeiodination of thyroxine in man, blocking the generation of metabolically active triiodothyronine ($T_3$) and favouring the formation of the metabolically inactive reverse $T_3$ ($rT_3$), was a further indication that amiodarone could possibly act through inhibiting the availability of the biologically active iodothyronine $T_3$. This hyopthesis was simultaneously tested by two groups. Sogol *et al.* (1983) compared the bradycardiac effect of amiodarone in rats to that of the iodinated contrast medium sodium ipodate which, like amiodarone, is a strong inhibitor of the thyroxine ($T_4$) $\rightarrow T_3$ conversion. The inhibition of this conversion step did not alter the heart rate.

At the same time, our own group (Lindenmeyer *et al.*, 1984) came to a similar conclusion by investigating, in guinea-pigs, the cardiac effect of iopanoic acid, another contrast medium, which is a potent inhibitor of the 5′ monodeiodination step (Bürgi *et al.*, 1976). We also postulated that the amiodarone effect on the heart rate was more likely to be the result of an

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interaction between amiodarone and triiodothyronine than from the inhibition of the local conversion of $T_4 \rightarrow T_3$ in the myocardium.

However, as it was found that inhibition of the $T_4 \rightarrow T_3$ conversion did not produce a bradycardia, it could not be concluded that the inhibition of this metabolic step does not prolong the action potential in contractile heart tissue, the key electrophysiological event for the anti-arrhythmic effect of amiodarone. Since the cellular uptake of $T_3$ from circulating blood seems to be much higher in the impulse conducting system than in the myocardium (Tomaselli *et al.*, 1965), the inhibition of the intracellular conversion of $T_4 \rightarrow T_3$ might have quite different consequences in these sites. In the present study we have, therefore, investigated whether the QT duration is changed by iopanoic acid-induced inhibition of the $T_4 \rightarrow T_3$ conversion.

The experiments were performed in guinea-pigs because repeated electrocardiographic recordings can be obtained without anaesthesia and the guinea-pig electrocardiogram is very similar to that of man.

## Methods

### *Study A*

Thirty-five male guinea-pigs were fed a breeding diet with an iodine content of $3\,mg\,kg^{-1}$ (Cat Nr 835 Nafag, Gossau, Switzerland). After a week, 3 groups were formed: 12 animals received $4\,g\,kg^{-1}$ iopanoic acid and 11 other animals were fed $12\,g\,kg^{-1}$ iopanoic acid. The sodium salt of iopanoic acid, kindly supplied by Leo AG, Zurich, Switzerland, was mixed into the food (4.0 g acid $kg^{-1}$ and 12 g acid $kg^{-1}$ respectively). Twelve animals, which served as controls, received no supplement. Electrocardiograms and body weight were registered at intervals of 6 to 8 days in all the animals during the period of feeding with contrast medium.

After 14 and 28 days of contrast medium feeding, animals of each group were killed by exsanguination under ether anaesthesia. Serum was frozen at $-20°C$ and samples of heart, liver and skeletal muscle taken for determination of tissue iodine content.

### *Study B*

After a week of feeding with the diet used in study A, 6 of 12 male guinea-pigs were given a supplement of $12\,g\,kg^{-1}$ iopanoic acid, while 6 were maintained on contrast medium-free food. ECG and weight recordings were performed as in study A, but recordings were made at intervals of 1 to 6 days in order to compare the initial time course of the QT duration and heart rate with that in study C.

### *Study C*

After 14 days of adaptation 4 guinea-pigs were given food containing $2.12\,g\,kg^{-1}$ amiodarone base. (Amiodarone was obtained from Sanofi Pharma AG, Basel, Switzerland, as tablets (Cordarone) and mixed with the food which was kept in the dark at 4°C). Four animals were used as controls. On day 7 of the amiodarone feeding period the animals were killed and blood and tissues processed as in study A.

### *Study D*

Food was restricted in two guinea-pigs for two months in order to achieve a weight loss. ECG recordings were performed as in studies A, B and C. Blood was taken by exsanguination under ether anaesthesia at the end of the study for determination of thyroid hormones.

### *Electrocardiography*

Leads I, II, III of the ECG were recorded in the physiological position of the animals without anaesthesia as described by Richtarik *et al.* (1965). A 3-channel apparatus with a paper velocity of $100\,mm\,s^{-1}$ was used. At least 10 RR intervals were recorded every minute for 3 min. The mean value of the heart rate was used for calculation of the mean value for a group of animals. The QT duration was measured on the three individual recordings and the mean taken for calculation of the mean value for the group.

### *Processing*

Heart muscle specimens were cleaned of superficial blood traces by suction with filter paper, weighed, homogenized and frozen at $-18°C$ until determination of iodine content.

### *Methods for measuring thyroid hormones and iodine*

Serum $T_4$ and reverse $T_3$ were measured using conventional RIA kits ($T_4$: Gamma Coat, Cat No CA-555, Clinical Assays, Cambridge, Massachusetts 02139, reverse $T_3$: RIA-mat $rT_3$, Byk-Mallinckrodt, Dietzenbach-Steinberg, FRG). Serum $T_3$ was not measured because, as shown earlier, it was consistently not detected in the serum of guinea-pigs (Lindenmeyer *et al.*, 1984).

The iodine content of the heart tissue was measured according to Lauber (1975). Determination of iodine is based on incineration of organic material followed by the redox reaction $2\,Ce^{IV} + As^{III} \rightleftharpoons 2\,Ce^{III} + As^{V}$, which is catalyzed by iodide and can be followed by photometric absorption. Both, inorganic iodide and organic iodine, including amiodarone- and iopanoic acid-iodine, were measured.

*Calculations*

Values are given as means ± 1 s.d. Standard statistical tests were used (Sachs 1974) and $P < 0.05$ was taken as the level of statistical significance.

## Results

*ECG and body weight*

*Study A* In control animals and animals given 4 g kg$^{-1}$ iopanoic acid the heart rate did not change (Figure 1).

In the group fed the higher dose of iopanoic acid the heart rate decreased from 274 min$^{-1}$ to 200 min$^{-1}$ (−27%) within two weeks ($P < 0.001$). After 4 weeks the heart rate was 197 min$^{-1}$ (−28%, $P < 0.001$ compared to the initial value). Compared with control animals the difference at this point in time was −25% ($P < 0.001$) and compared with animals given the low contrast medium dose it was −31% ($P < 0.001$).

The QT duration remained unchanged in controls. In animals fed the lower dose of iopanoic acid, it decreased from 122 to 109 ms (−11%, $P < 0.01$) within 2 weeks but the value did not differ from the respective value of the control group and it increased again to the initial value after 4 weeks of treatment.

In animals treated with the higher dose of iopanoic acid, the QT duration increased from 116 to 141 ms (+22%) within two weeks ($P < 0.001$) and increased by another 9 ms in the following 2 weeks, the total increase within 4 weeks amounting to +29% of the initial value ($P < 0.001$). At this point in time the QT duration was +25% ($P < 0.001$) compared to controls and +30% compared to animals given the lower dose of iopanoic acid ($P < 0.001$).

*Body weight* The weight of control animals and animals fed the lower dose of iopanoic acid increased steadily. In animals fed the higher dose of iopanoic acid the body weight decreased by 28% ($P < 0.001$) within 4 weeks.

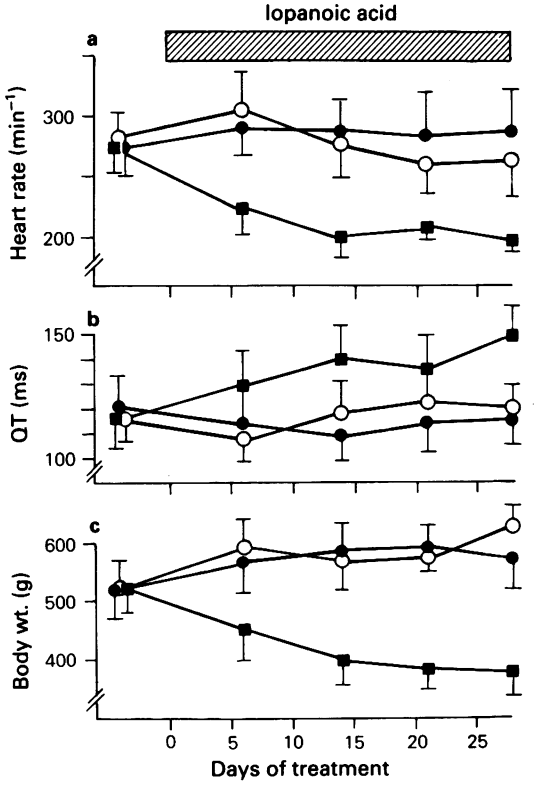


**Figure 1** (a) Heart rate, (b) QT duration and (c) body weight in guinea-pigs from study A. The points represent mean values ± s.d. of 5 to 12 animals. (○) Controls; (●) 4 g kg$^{-1}$; (■) 12 g kg$^{-1}$ iopanoic acid.

*Study B* Alterations of ECG and body weight were identical to those in study A. In addition ECG recordings performed within the first days after the beginning of iopanoic acid feeding showed that there was a lag period of 3 to 6 days between the beginning of high-dose iopanoic acid treatment and the ECG alterations (Figure 2A).

*Study C* Within 6 days after the beginning of amiodarone treatment the heart rate decreased by 20% ($P < 0.01$), most of this decrease occurred within the first 2 days. The QT duration increased by 18%. Heart rate and QT duration did not change in the control group. In contrast to the animals fed the high dose of iopanoic acid, the weight of amiodarone-treated animals increased (Figure 2B).

*Study D* After a weight loss of 32% and 35% in the two food-restricted animals the QT duration was prolonged by 6% and 12%, and the heart rate decreased by 5% and 19%. The $T_4$ concentrations of 16 nmol l$^{-1}$ and 14 nmol l$^{-1}$ were below the normal range while the respective reverse $T_3$ concentrations of 59 pmol l$^{-1}$ and 58 pmol l$^{-1}$ were within the normal range. These changes were expected to occur in starved animals (Chopra, 1981).

*Thyroid hormones*

*Study A* After 2 weeks of feeding with iopanoic acid, $T_4$ was 100% higher in animals administered the high dose compared to controls and 180% higher in animals given the low dose compared to controls

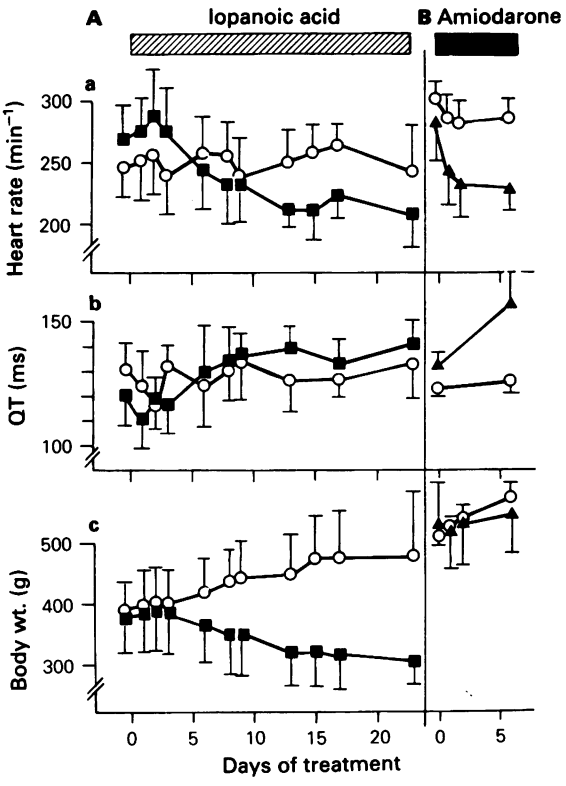


**Figure 2** (a) Heart rate, (b) QT duration and (c) body weight in guinea-pigs from studies B and C. The points from study B (A) represent mean values ± 1 s.d. of 6 animals, the points from study C (B) are means ± s.d. of 4 animals. (○) Controls; (■) 12 g kg$^{-1}$ iopanoic acid; (▲) 2.12 g kg$^{-1}$ amiodarone.

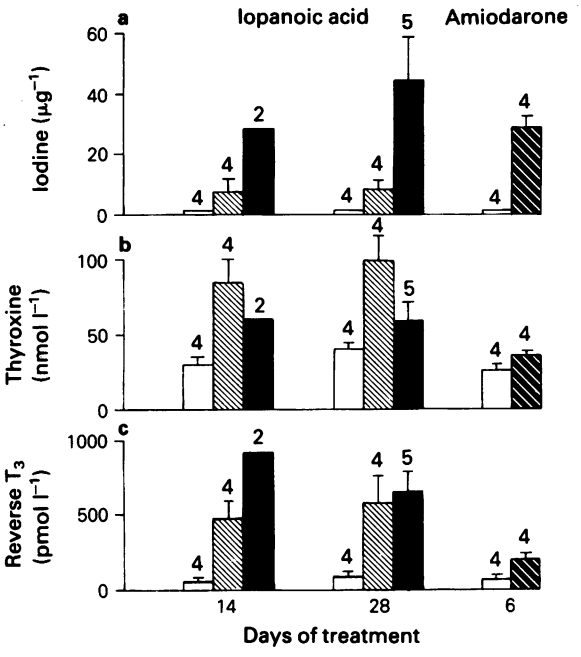


**Figure 3** (a) Iodine concentrations in the heart and serum (b) thyroxine and (c) triiodothyronine concentrations, in animals killed in studies A and C after different periods of treatment with iopanoic acid and amiodarone. The heart iodine concentration in animals treated with 12 g kg$^{-1}$ iopanoic acid was virtually the same as in amiodarone-treated animals whereas reverse $T_3$ concentrations were largely different in these groups. Open columns represent controls; hatched columns, 4 g kg$^{-1}$ iopanoic acid; solid columns, 12 g kg$^{-1}$ iopanoic acid; hatched solid columns, 2.12 g kg$^{-1}$ amiodarone.

($P<0.01$) (Figure 3). After 4 weeks the respective differences were +48% and +148% ($P<0.01$).

The expected increase of reverse $T_3$ reflecting the inhibition of the $T_4 \rightarrow T_3$ conversion, was also demonstrated: after 2 weeks reverse $T_3$ was 1,410% above controls ($P<0.01$) in animals administered the high dose and 710% above controls ($P<0.01$) in animals fed the low dose. After 4 weeks the respective differences were +578% and +504% ($P<0.01$ compared to controls). There was no longer any difference between the animals fed the high and the low dose of contrast medium.

*Study C* In the amiodarone group $T_4$ was 35% above ($P<0.01$) and reverse $T_3$ was 200% above ($P<0.01$) the value in the control group (Figure 3).

*Iodine concentration in heart muscle*

*Studies A and C* Figure 3 shows that the iodine concentration in heart was higher in animals fed contrast medium and amiodarone than in controls. After 2 weeks of iopanoic acid treatment the heart iodine content of animals fed the high dose was 282% above that of animals given the low dose. After 4 weeks the respective difference was +424% ($P<0.02$). The iodine concentration in the heart of amiodarone fed animals was not significantly different ($P>0.05$) from that of animals given the high dose of contrast medium for 2 and 4 weeks. The iodine concentration was higher in the heart of amiodarone-treated animals than in animals given the low dose of iopanoic acid ($P<0.001$).

## Discussion

In the present study we came to the conclusion that the diminished peripheral generation of the metabolically most active iodothyronine $T_3$, which invariably follows amiodarone administration is in no way causally related to the prolongation of the action

potential in the heart muscle. The findings expand earlier conclusions based on the bradycardiac potency of amiodarone. The experiments were mainly founded on a comparison of the known action of amiodarone with that of iopanoic acid, a specific and potent inhibitor of the generation of $T_3$ from $T_4$.

With the low dose of iopanoic acid a strong inhibition of the $T_4 \rightarrow T_3$ conversion was present after 14 days and 28 days of treatment. This is evident as an increase in $T_4$ to between 2 and 3 times the control value and an increase of the metabolically inactive reverse $T_3$ to between 6 and 7 times the control value (Chopra, 1981). These effects could not be further enhanced by increasing the dose of iopanoic acid. In spite of the already maximal inhibition of the deiodination at the outer ring of the thyroxine molecule, a prolongation of the QT duration was not achieved. Furthermore, a decrease in heart rate was not observed, confirming earlier results (Sogol *et al.*, 1983; Lindenmeyer *et al.*, 1984). In contrast, QT prolongation and reduction of the heart rate occurred as early as six days after the start of amiodarone treatment and at concentrations of $T_4$ and reverse $T_3$ considerably less elevated than those in animals treated with iopanoic acid (Figure 3).

It can, therefore, be concluded that the inhibition of the $T_4 \rightarrow T_3$ conversion alone is not the key event for either the QT prolongation or the decrease of heart rate observed during amiodarone treatment.

The argument that the local inhibition of $T_3$ generation in myocardial tissue was perhaps less pronounced in iopanoic acid-treated animals than in amiodarone-treated animals, because of a lower drug concentration, is invalidated by the finding that iopanoic acid is a much stronger inhibitor of the $T_4 \rightarrow T_3$ conversion than amiodarone. The absence of even a slight QT prolongation in iopanoic acid-treated animals clearly indicates that the inhibition of $T_4 \rightarrow T_3$ conversion alone does not account for this electrophysiological effect.

Amiodarone not only inhibits the $T_4 \rightarrow T_3$ conversion step but may also interfere with the effects of $T_3$ (Lindenmeyer *et al.*, 1984) and has $\alpha$- and $\beta$-adrenoceptor blocking properties (Charlier *et al.*, 1967). It is possible, therefore, that the action of amiodarone on the ECG is due to a combination of these effects. At a dose of 4 g $kg^{-1}$, iopanoic acid may have no effect on the ECG, because its action is limited to only one of the various effects of amiodarone, i.e. the block of $T_4 \rightarrow T_3$ conversion.

The increase of the dose of iopanoic acid from 4 to 12 g $kg^{-1}$ was associated with a further accumulation of the drug in the heart muscle, which was eventually similar to the concentration of amiodarone in terms of iodine content of the two compounds. This was associated with a gradual QT prolongation and decrease of the heart rate. Since the concentrations of $T_4$ and reverse $T_3$ were still identical to those observed with the lower dose of iopanoic acid, the electrophysiological changes again cannot be a consequence of alterations in thyroid hormone metabolism alone. At least two other reasons for the changes in QT have to be considered: (1) it is possible that iopanoic acid has an intrinsic effect similar to the electrophysiological effect of amiodarone if its concentration in the myocardium is high enough. (2) The QT prolongation and the decrease of the heart rate may, more likely, be causally related to the associated weight loss of the animals indicating frank toxicity of the high dose of iopanoic acid. This is suggested by the prolongation of the QT duration and the decrease of the heart rate in two animals fed a restricted diet alone. The comparison between animals under iopanoic acid treatment and food-restricted animals is, however, hampered by the finding that $T_4$ concentrations were elevated in the former but decreased in the latter. It remains uncertain at the present time, which of the many effects of starving, including diminished availability of $T_3$, ultimately accounts for the electrophysiological changes occurring in the heart muscle.

The present findings indicate, that the inhibition of the $T_4 \rightarrow T_3$ conversion is not the decisive event in the amiodarone-induced prolongation of the refractory period of the ECG in guinea-pigs. They do not exclude the possibility that iopanoic acid has intrinsic effects on QT duration and heart rate, but they provide additional evidence for the view (Meese *et al.*, 1985) that the impressive action of amiodarone on heart muscle is probably not mediated by the block of the $T_4 \rightarrow T_3$ conversion alone.

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