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Does amiodarone affect heart rate by inhibiting the intracellular generation of triiodothyronine from thyroxine?

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1 The hypothesis that the antiarrhythmic drug amiodarone slows down the heart rate by its inhibitory action on the intracellular conversion of thyroxine (T_4) to 3,5,3′ triiodothyronine (T_3) was investigated. For this purpose we compared the effect of amiodarone with that of another potent inhibitor of the $T_4 \rightarrow T_3$ conversion, i.e. the radiographic contrast medium iopanoic acid, on the heart rate of unanaesthetized guinea-pigs.

2 Both amiodarone and, to an even greater extent, iopanoic acid induced an increase in serum 3.5',3' triiodothyronine (reverse T_3), indicating effective inhibition of $T_4 \rightarrow T_3$ conversion. Both amiodarone and iopanoic acid were accumulated in the liver and in the heart (measured as iodine).

3 While amiodarone induced bradycardia, iopanoic acid did not change the heart rate.

4 Supraphysiological amounts of exogenous T_3 reverted the amiodarone induced bradycardia to near normal values. A comparable effect was observed with isoprenaline.

5 The intracellular inhibition of the $T_4 \rightarrow T_3$ conversion is not the ultimate mode of the action of the amiodarone effect on heart rate. It is thought that amiodarone interacts with T_3 at its receptor or somewhere later along the pathway from the T_3-receptor interaction to the final effect of T_3 on heart rate.

Introduction

Amiodarone, a derivative of benzofurane, containing 39% iodine, was originally used as an anti-anginal agent (Vastesaeger *et al.*, 1967; Leutenegger & Lüthi, 1968). The drug has recently attracted wide interest because of its powerful anti-arrhythmic properties (Van Schepdael & Solvay, 1970; Rosenbaum *et al.*, 1976; Heger *et al.*, 1981). The mechanism of action appears to be different from that of most if not all other anti-arrhythmics and has yet to be clarified. Since some of the effects of amiodarone on the heart resemble those of hypothyroidism (Singh & Vaughan Williams, 1970; Freedberg *et al.*, 1970) and since amiodarone does indeed interfere with thyroid function in various ways, Singh & Vaughan Williams have investigated, in rabbits and guinea-pigs, the possibility that amiodarone may dampen arrhythmogenic foci and cause bradycardia through its inhibitory action on the thyroid. Although in these experiments some effects of amiodarone could be overridden by simultaneous treatment with thyroxine (T_4), amiodarone did not cause any depression of thyroid function. The authors (Singh & Vaughan Williams, 1970), therefore, considered the possibility that amiodarone interfered with some effects of thyroxine on the heart. However, the problem remained open and no supporting new evidence in favour of this hypothesis was produced.

A new area of research opened when Burger *et al.*, (1976) demonstrated that amiodarone was a powerful inhibitor of the extrathyroidal conversion of T_4 to triiodothyronine (T_3). Following this observation, we reviewed the existing evidence deemed compatible with the idea that amiodarone may in fact act at the cellular level by inhibiting the intracellular generation of the metabolically active thyroid hormone 3,5,3′ triiodothyronine (T_3) from its precursor T_4, and that this occurred predominantly within the impulse conducting and generating system of the heart (Singh & Vaughan Williams, 1970; Melmed *et al.*, 1981). It was even proposed that the antiarrhythmic effect of amiodarone may parallel the increasing serum concentration of 3,5′,3′ T_3 or reverse T_3 generated in response to a block of the enzyme T_4-5′ deiodinase (Nademanee *et al.*, 1982). Intracellularly generated T_3, rather than blood T_3, has previously been shown to be by far the most important source of

metabolically active hormone at the level of the pituitary gland (Silva & Larsen, 1977). Therefore, in the present experiments, guinea-pigs were fed amiodarone in doses large enough to produce bradycardia along with the expected inhibition of T_3 generation from T_4. Another group of animals was treated with iopanoic acid, a radiographic agent, which is among the most potent inhibitors of the conversion of T_4 to T_3 (Bürgi *et al.*, 1976). The question was, whether this agent would, like amiodarone, also induce bradycardia and if so, whether bradycardia could be prevented by simultaneous application of exogenous T_3? The application of exogenous T_3 circumvents the need for intracellular generation of T_3 from T_4.

Methods

Two sets of experiments were performed: (1) In 44 male guinea-pigs (mean weight 430 g, group A) the effect of amiodarone on the heart rate was compared with that of iopanoic acid. (2) Sixteen more guinea-pigs (mean weight 411 g, group B) were used to test the effect of T_3 and isoprenaline on amiodarone induced bradycardia.

The animals were fed a breeding diet with an iodine content of $3\,mg\,kg^{-1}$ (Cat. No 835 Nafag, Gossau, Switzerland). Amiodarone base $4.24\,g\,kg^{-1}$, day 0 to 25 and $1.41\,g\,kg^{-1}$, day 26 to 50 were added to the food of group A, and 2.12 g amiodarone base per kg to the food of group B. 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. With the higher dosage ($4.24\,g\,kg^{-1}$ food) the animals stopped growing. This was taken to indicate the upper level of tolerance.

The sodium salt of iopanoic acid, kindly supplied by Leo AG, Zürich, Switzerland, was mixed into the food ($4.0\,g\,acid\,kg^{-1}$). Pure T_3 (Fluka AG, Buchs, Switzerland) was injected subcutaneously daily in doses of 20 μg. This clearly supraphysiological dose invariably stopped the growth of the guinea-pigs. The same volume (0.2 ml) of 0.9% w/v NaCl solution (saline) was injected subcutaneously into control animals.

ECG recording

ECGs were recorded in the physiological position of the animals without anaesthesia as described by Richtarik *et al.*, (1965). Measurements were performed immediately before the daily injections of T_3 and saline. At least 10 RR-intervals were recorded in each animal for determination of the heart rate.

Methods for measuring thyroxine (T_4), triiodothyronine (T_3), reverse T_3 and iodine

The animals were killed by aortic exsanguination under ether anaesthesia. Serum T_4 and T_3 were measured using conventional RIA kits. (T_4: Gamma Coat, Cat. No. CA–555, Clinical Assays, Cambridge, Massachusetts 02139, T_3: Gamma Coat, Cat. No. CA–561, Clinical Assays, Cambridge, Massachusetts 02139, rT_3: RIA-mat rT_3, Byk-Mallinckrodt, Dietzenbach – Steinberg, FRG.)

T_3 was consistently undetectable in the serum of guinea-pigs. However, when 20 μg of T_3 was injected subcutaneously T_3 became easily measurable and rose to peak levels of $28\,nmol\,l^{-1}$ within 2 h. Therefore, the very low endogenous T_3 levels are real, not due to insensitivity of the RIA used.

Iodine Iodine was determined in heart and liver according to Lauber (1975), a method which has been used in this laboratory for previous studies (Studer *et al.*, 1978; Stäubli *et al.*, 1983). In principle, 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 which can be measured by photometric absorption. Both inorganic iodide and organic iodine, including amiodarone iodine, are measured.

Isoprenaline treatment

Isoprenaline ($5\,\mu g\,100\,g^{-1}$ body weight) was injected intraperitoneally in amiodarone pretreated animals. The heart rate was recorded before and 1, 3, 5, 7 and 11 min after injection. The maximal increase in heart rate was taken as an index of effectiveness.

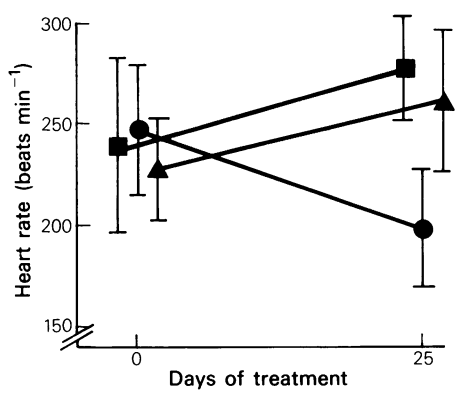

Figure 1 Heart rate of guinea-pigs before and after 25 days of treatment with iopanoic acid (▲) ($n = 16$), and amiodarone (●) ($n = 14$) as compared to controls (■) ($n = 9$).

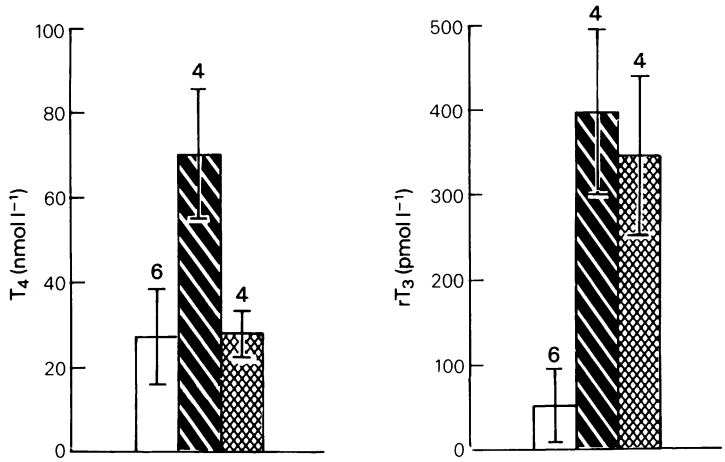

Figure 2 Serum thyroxine (T_4) and 3,5',3' triiodothyronine (rT_3) after 25 days of treatment with iopanoic acid 4 g kg^{-1} food (solid, diagonally hatched columns) and with amiodarone 4.24 g kg^{-1} food (hatched columns) as compared to control animals (open columns). The number of animals is indicated above the columns.

Calculations

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

Results

Heart rate

With the higher dose of amiodarone (4.24 g kg^{-1} food) the heart rate decreased from 247 ± 32 min^{-1} ($n = 14$) to 199 ± 29 min^{-1} (−19%) after 25 days ($P < 0.01$) (Figure 1). With the lower dose (1.41 g kg^{-1} food) given for another 25 days it returned to the initial value (247 ± 32 min^{-1}) by day 50. Amiodarone at a dose of 2.12 g kg^{-1} food invariably induced bradycardia in guinea-pigs, an effect already apparent within 1 day. This was confirmed in many more experiments not included here.

In animals treated with iopanoic acid the heart rate increased from 228 ± 25 min^{-1} ($n = 16$) to 262 ± 35 min^{-1} (+15%) after 25 days ($P < 0.001$) (Figure 1) and returned to the initial value by day 50 (227 ± 30 min^{-1}). A similar course was observed in the control group: the heart rate rose from 240 ± 43 min^{-1} ($n = 9$) to 278 ± 26 min^{-1} after 25 days ($P < 0.05$) (Figure 1) and declined thereafter to 244 ± 25 min^{-1} by day 50.

Serum thyroxine (T_4) and reverse triiodothyronine (rT_3)

Figure 2 demonstrates that on day 25, T_4 had risen by 206% as compared to the initial value ($P < 0.001$) only in iopanoic acid-treated animals but had not yet changed in amiodarone-treated animals ($P > 0.2$). It was only when amiodarone treatment was continued for another 25 days at a dose of 1.41 g kg^{-1} food that the expected increase became measurable. Indeed, in this case T_4 rose from an initial value of 23.0 ± 6.4 nmol l^{-1} to 48.6 ± 7 nmol l^{-1}, i.e. $\Delta T_4 = +111\%$ ($P < 0.01$). The earlier increase of absolute T_4 values observed with iopanoic acid indicates that the deiodination pathway of T_4 is more efficiently blocked with this agent than with amiodarone (Chopra, 1981). Reverse T_3 also increased to a somewhat higher level with iopanoic acid (398 ± 95 p mol l^{-1}, $n = 4$) than with amiodarone (347 ± 94 p mol l^{-1}, $n = 4$), corresponding to a change of +588% and +690%, as related to the initial value of 50 ± 16 p mol l^{-1}; the difference in the increase was, however, not statistically significant ($P > 0.20$).

The difference became significant ($P < 0.001$) when treatment was continued for another 25 days with 1.41 g amiodarone kg^{-1}. In this case, reverse T_3 in amiodarone-treated animals decreased to 165 ± 26 p mol l^{-1} ($n = 5$), corresponding to a 228% increase compared to the initial value of 50 ± 16 p mol l^{-1} ($n = 5$) ($P < 0.001$). With iopanoic acid, however, reverse T_3 further increased to 478 ± 124 p mol l^{-1} ($n = 6$), corresponding to an increase of 848% compared to the initial value of 50 ± 16 p mol l^{-1} ($n = 5$), ($P < 0.001$). The difference of 313 p mol l^{-1} between reverse T_3 in animals treated with amiodarone and iopanoic acid was highly significant ($P < 0.001$).

Since by day 50 of this regime the heart rates of the two groups were virtually identical (247 ± 32 min^{-1}

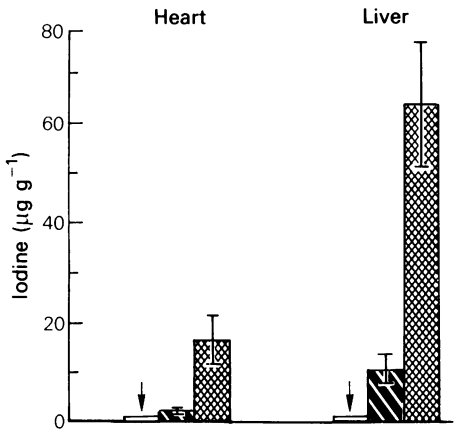

Figure 3 Iodine content of organs in guinea-pigs treated with iopanoic acid 4 g kg^{-1} food for 50 days (solid, diagonally hatched columns) ($n=5$) and amiodarone 4.24 g kg^{-1} food for 25 days followed by 1.41 kg up to 50 days (cross hatched columns) ($n=6$) as compared to controls (open columns) ($n=2$). →below detectable levels i.e. <1 μg g^{-1} material.

and 227 ± 30 min^{-1}) and no longer different from those recorded before treatment we concluded that even the highly effective inhibition of T_4-5' deiodinase in iopanoic acid-treated animals fails to induce bradycardia.

Organic iodine in liver and heart

Figure 3 indicates that large amounts of organic iodine were accumulated in both the liver and the heart in response to amiodarone as well as to iopanoic acid treatment. Six to eight times more iodine was found in both organs after amiodarone than after iopanoic acid treatment. In both instances approximately 5 times more iodine was found in the liver than in the heart.

Effect of isoprenaline on normal and amiodarone slowed heart rate

Table 1 indicates that isoprenaline increases the heart rate in normal guinea-pigs and that it does even more so in T_3 pretreated guinea-pigs. T_3-induced thyrotoxicosis (as indicated by an arrest of weight gain) was not severe enough to produce tachycardia by itself, although it sensitized the heart to the β-stimulating drug isoprenaline.

In amiodarone-treated guinea-pigs, isoprenaline caused the same relative increase in heart rate as in controls. However, the heart rate still remained considerably below that in normal controls (Table 1).

Table 1 Effect of isoprenaline on heart rate in 3,5,3′ triiodothyronine (T_3) and amiodarone treated guinea-pigs (group B)

	Heart rate before isoprenaline	*Maximum heart rate within 11 min after isoprenaline*	*% increas*
Controls ($n=4$)	283 ± 21	364 ± 21‡	28.6
T_3 treated* ($n=3$)	302 ± 19	463 ± 14§	51.7
Amiodarone treated† ($n=4$)	222 ± 18	272 ± 20‖	22.5

* T_3 was injected subcutaneously 20 μg per day for 19 days.
† Amiodarone was given in the food (2.12 g kg^{-1}) for 19 days.
‡ $P<0.02$, § $P<0.01$, ‖ $P<0.05$, as compared to values before isoproterenol.

Effect of triiodothyronine (T_3) on amiodarone induced bradycardia

Figure 4 indicates that amiodarone induced bradycardia can be overcome by high doses of T_3. After cessation of T_3 treatment the heart returns to its slow pace characteristic of amiodarone treatment alone. The mean increase in heart rate caused by T_3 was 12.5% ($n=4$, $P<0.02$). On repeating the injection of T_3 after 41 days of amiodarone treatment in the same animals the increase in heart rate was 11.5% ($P<0.01$). Saline injection did not change the heart rate in control animals ($n=4$, $P>0.5$).

Discussion

Amiodarone treatment of guinea-pigs for 25 days reduced the heart rate by 19%. Simultaneously the serum concentration of reverse T_3 rose by 588%, indicating an impaired generation of intracellular T_3 by inhibition of T_4 deiodination at the outer ring with enhancement of T_4 deiodination at its inner ring (Chopra, 1981). The same effect, although quantitatively so much more pronounced that the inhibition of T_4 deiodination resulted not only in an even more increased formation of reverse T_3 but also in a rise in serum T_4 within 25 days, was brought about by iopanoic acid. However, this agent did not decrease heart rate. It therefore appears that the inhibition of intracellular generation of T_3 from T_4 is not the ultimate mode of action of amiodarone. It could be argued that amiodarone may be accumulated selectively in the heart while iopanoic acid is not. However, iopanoic acid also accumulates in the heart. It is

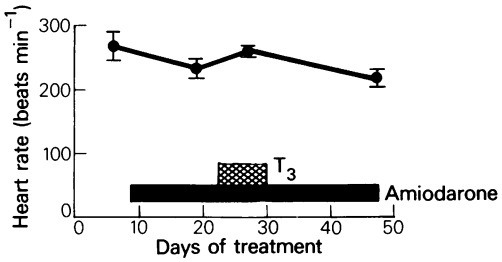

Figure 4 Heart rate of 4 guinea-pigs before and during amiodarone treatment and during combined treatment with amiodarone and 3,5,3′ triiodothyronine (T_3). Each point represents the mean value of measurements for 3 days.

true that 6 to 8 times more amiodarone is taken up (on the basis of the weight of tissular iodine) by the heart and by the liver in amiodarone-treated animals compared to iopanoic acid-treated animals. However, since iopanoic acid is a more efficient blocker of 5′ tetraiodothyronine deiodinase, the total absence of any decrease in heart rate induced by this compound may be safely taken to indicate that the block of intracellular thyroxine deiodination is not an essential part of the action of amiodarone on the heart rate.

The amount of iodine accumulated in the liver in response to both amiodarone and iopanoic acid is near that usually found in the thyroid. Yet the thyroid contains up to 10% of thyroglobulin, a unique protein capable of being iodinated to a level of about 0.5 to 1%. A current figure for the iodine content of a normal thyroid is $300\,\mu g\,g^{-1}$ which compares to the $60\,\mu g\,g^{-1}$ found in the guinea-pig liver in the present study. It is not known what kind of structure these excessive amounts of iodine are bound to in organs not containing any known protein designed to store iodine.

If the inhibition of intracellular T_3 generation is not the mechanism by which amiodarone slows down the heart rate, the drug could still interact with T_3 at its receptor or somewhere later along the process from the T_3 receptor interaction to the final effect of T_3 on the heart rate. This seems indeed to be the case, since exogenous T_3, not dependent on intracellular generation from T_4, at least partly reverses the amiodarone-induced bradycardia. Thus, amiodarone creates, in the heart, a condition opposite to that normally prevailing in the pituitary gland, where the regulation of the TSH-feedback system mainly depends on intracellularly generated T_3 and not on circulating T_3 (Silva & Larsen, 1977).

Like exogenous T_3, isoprenaline also partly reverses the amiodarone-induced bradycardia. Therefore, amiodarone must have a very peculiar action on the impulse forming system of the heart, since it seems to have a desensitizing effect on several pharmacological agents which have different mechanisms of action. It is not surprising that amiodarone has recently been shown to possess, besides its weak α- and β-blocking properties (Charlier *et al.*, 1967; Polster & Broekhuysen, 1976), a slow-calcium-channel blocking action (Gloor *et al.*, 1983). The ultimate mode of action of the potent antiarrhythmic drug amiodarone still remains far from clear.

The present experiments only rule out the possibility that amiodarone effects the guinea-pig heart rate by inhibiting intracellular T_3 generation from T_4 in the impulse generating system. They indicate that the drug interacts directly with the metabolic effect of extracellular, circulating T_3.

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Note added in proof: Very recently, Sogol *et al.* (*Endocrinology* 1983, **113,** 1464–1469) published a report on the effects of amiodarone and sodium ipodate on the heart rate of rats, which is in agreement with our observations in guinea-pigs.

References

BUERGI, H., WIMPFHEIMER, C., BURGER, A., ZAUNBAUER, W., ROESLER, H. & LEMARCHAND-BERAUD, T. (1976). Changes of circulating thyroxine triiodothyronine and reverse triiodothyronine after radiographic contrast agents. *J. clin. Endocrinol. Metab.*, **43,** 1203–1210.

BURGER, A., DINICHERT, D., NICOD, P., JENNY, M., LEMARCHAND-BERAUD, T. & VALLOTTON, M.B. (1976). Effect of amiodarone on serum triiodothyronine, reverse triiodothyronine, thyroxine and thyrotropin. *J. clin. Invest.*, **58,** 255–259.

CHARLIER, R., BAUDINE, A. & CHAILLET, F. (1967). Recherches dans la série des benzofurannes. XXXII. Mode d'action de l'amiodarone sur le système cardiovasculaire. *Arch. int. Physiol. Biochim.*, **75,** 787–808.

CHOPRA, I.J. (1981). *Triiodothyronines in health and disease.* Berlin, Heidelberg and New York: Springer-Verlag.

FREEDBERG, A.S., PAPP, J.G. & VAUGHAN WILLIAMS, E.M. (1970). The effect of altered thyroid state on atrial intracellular potentials. *J. Physiol.*, **207,** 357–369.

GLOOR, H.O., URTHALER, F. & JAMES, T.N. (1983). Acute

effects of amiodarone upon canine sinus node and atrioventricular junctional region. *J. clin. Invest.*, **71,** 1457–1466.

HEGER, J.J., PRYSTOWSKY, E.N., JACKMAN, W.M., NACCARELLI, G.V., WARFEL, K.A., RINKENBERGER, R.L. & ZIPES, D.P. (1981). Amiodarone. Clinical efficacy and electrophysiology during long-term therapy for recurrent ventricular tachycardia or ventricular fibrillation. *N. Engl. J. Med.*, **305,** 539–545.

LAUBER, K. (1975). Iodine determination in biological material. Kinetic measurement of the catalytic activity of iodide. *Anal. Chem.*, **47,** 769–771.

LEUTENEGGER, A. & LUETHI, E. (1968). Eine neue Substanz in der Therapie der Angina pectoris: Amiodarone. *Schweiz. Med. Wochenschr.*, **98,** 2020–2025.

MELMED, S., NADEMANEE, K., REED, A.W., HENDRICKSON, J.A., SINGH, B.N. & HERSHMAN, J.M. (1981). Hyperthyroxinemia with bradycardia and normal thyrotropin secretion after chronic amiodarone administration. *J. clin. Endocrinol. Metab.*, **53,** 997–1001.

NADEMANEE, K., SINGH, B.N., HENDRICKSON, J.A., RED, A.W., MELMED, S. & HERSHMAN, J. (1982). Pharmacokinetic significance of serum reverse T_3 levels during amiodarone treatment: a potential method for monitoring drug therapy. *Circulation.*, **66,** 202–211.

POLSTER, P. & BROEKHUYSEN, J. (1976). The adrenergic antagonism of amiodarone. *Biochem. Pharmac.*, **25,** 131–134.

RICHTARIK, A., WOOLSEY, T.A. & VALDIVIA, E. (1965). Method for recording ECGs in unanesthetized guinea pigs. *J. appl. Physiol.*, **20,** 1091–1093.

ROSENBAUM, M.B., CHIALE, P.A., HALPERN, M.S., NAU, G.J., PRZYBYLSKI, J., LEVI, R.J., LAZZARI, J.O. & ELIZARI, M.V. (1976). Clinical efficacy of amiodarone as an antiarrhythmic agent. *Am. J. Cardiol.*, **38,** 934–944.

SACHS, L. (1974). *Angewandte Statistik.* Berlin, Heidelberg and New York: Springer-Verlag.

SILVA, J.E. & LARSEN, P.R. (1977). Pituitary nuclear 3,5,3′-triiodothyronine and thyrotropin secretion: an explanation for the effect of thyroxine. *Science*, **198,** 617–620.

SINGH, B.N. & VAUGHAN WILLIAMS, E.M. (1970). The effect of amiodarone, a new anti-anginal drug, on cardiac muscle. *Br. J. Pharmac.*, **39,** 657–667.

STAEUBLI, M., BIRCHER, J., GALEAZZI, R.L., REMUND, H. & STUDER, H. (1983). Serum concentrations of amiodarone during long-term therapy. Relation to dose, efficacy and toxicity. *Eur. J. clin. Pharmac.*, **24,** 485–494.

STUDER, H., FORSTER, R., CONTI, A., KOHLER, H., HAEBERLI, A. & ENGLER, H. (1978). Transformation of normal follicles into thyrotropin-refractory 'cold' follicles in the aging mouse thyroid gland. *Endocrinology*, **102,** 1576–1586.

VAN SCHEPDAEL, J. & SOLVAY, H. (1970). Etude clinique de l'amiodarone dans les troubles du rhythme cardiaque. *Presse Med.*, **78,** 1849–1850.

VASTESAEGER, M., GILLOT, P. & RASSON, G. (1967). Etude clinique d'une nouvelle médication antiangoreuse. *Acta Cardiol. (Brux.)*, **22,** 483–500.

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