The Effect of Hexobendine on the Function and Metabolism of the Isolated, Perfused Rat Heart*

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SUMMARY

The effect of hexobendine (Ustimon R) on the function and metabolism of the isolated, perfused rat heart has been studied. Hexobendine produces a significant negative chronotropic effect, which may account for the significant reduction both in substrate uptake and metabolism and in function. Aerobic metabolism was not inhibited by hexobendine. A significant negative inotropic effect produced by hexobendine was also observed.

In the light of the present study, it seems possible that hexobendine relieves angina pectoris by virtue of its negative chronotropic effect, thereby reducing the oxygen consumption of the heart.

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Hexobendine (Ustimon R), a new anti-angina substance (not related to the nitrates), has been used with success in the long-term treatment of angina pectoris.[1] Previous studies on the isolated rat and rabbit heart, as well as on the intact anaesthetized dog[2-4] showed that hexobendine increased coronary blood flow and decreased myocardial oxygen consumption. Since treatment of angina pectoris is aimed at an increase in coronary blood flow and/or a decrease in myocardial oxygen demands, it seems as if hexobendine exerts its anti-angina effect by the above-mentioned changes.

However, the exact mechanism of the action of hexobendine has not yet been established. Therefore a study was made of the effect of hexobendine on the function, coronary flow rate, oxygen consumption and substrate metabolism of the isolated, perfused rat heart.

MATERIAL AND METHODS

Female albino rats (Wistar strain) weighing about 200 g were used. All animals were fed *ad libitum* until decapitation.

Metabolic Studies

The isolated rat heart was perfused with 15 ml of a modified Krebs-Henseleit bicarbonate buffer (pH 7·4)[5] for 30 minutes in a modified Langendorff perfusion system as outlined before.[6] [U-^{14}C] glucose (10 mM), [1-^{14}C] palmitate (0·7 mM) and [3-^{14}C] pyruvate (6 mM) (obtained from the Radiochemical Centre, Amersham, England) were used as substrates.

Determinations and calculations of glucose uptake, lactate production, pyruvate uptake or production, titratable and ^{14}C-fatty acid uptake, incorporation of ^{14}C-palmitate into tissue lipids and ^{14}C-glucose into cardiac glycogen and residual cardiac glycogen content were carried out as previously described.[6,7]

For determination of myocardial high-energy phosphate contents, the hearts were clamped after perfusion with Wollenberger tongs, pre-cooled in liquid nitrogen. Myocardial adenosine triphosphate content (ATP) was determined enzymatically.[8] The myocardial adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were determined, using the method of Adam.[8] Creatine phosphate (CrP) and inorganic phosphate (Pi) contents were determined according to the method of Furchgott and De Gubareff.[9] Myocardial oxygen uptake and coronary flow rate were determined, as described previously.[10]

To eliminate the significant decrease in heart rate caused by hexobendine, substrate metabolism and oxygen consumption were also studied in a series of rat hearts treated with hexobendine, and paced at a constant rate of ±200 beats/min by a Medronic R-Wave-coupled Pulse Generator (Model 5837) to approach the average rate of the control isolated beating heart.

Mechanical Studies

The mechanical activity of the perfused rat heart was determined, using a modified Langendorff perfusion system and a differential myographic force transducer.[11] The hearts were perfused with 30 ml of perfusate, containing 10 mM glucose as substrate. The isolated beating heart was preloaded with a 4 g weight and perfused at a constant pressure of 60 mmHg over a period of 45 minutes.[11]

Measurements of work performance included the determination of peak height of developed tension (PH) in millimetres, the tension time index (TTI) in milligram-seconds, obtained by planimetric integration of the area under the systolic curve, and also tension time/min (TTM).[12] The maximum rate of rise of developed tension (DT/dt_{MAX}) in milligram-seconds, and the time to peak height of developed tension (t-PH) in microseconds, were also measured. The degree of stress relaxation (SR) as measured by the increase of resting length was recorded.[11] The above parameters were measured at 5-minute intervals.

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In order to eliminate the effect of reduced heart rate on the mechanical performance, the hearts were stimulated with a pacemaker to maintain control rates (±200 beats/min) and the mechanical activity was measured.

In all experiments hexobendine was added to the perfusate in a concentration of 5 μg/ml perfusate. All results are expressed as means ± SEM (number of observations). *P* values are derived from Student's *t*-test.[13]

RESULTS

Effect of Hexobendine on Metabolic Patterns

Hexobendine caused a significant reduction in heart rate throughout the experiments (control 200 ± 3; hexobendine-treated hearts 136 ± 6).

Hexobendine reduced myocardial glucose uptake, $^{14}CO_2$ and lactate production, while pyruvate production, [^{14}C] glucose incorporation into glycogen and residual glycogen content were unaffected. Increasing the heart rate with a pacemaker to average control rates (200 ± 3), had no effect on glucose uptake and $^{14}CO_2$ production, while lactate production was significantly increased. Although the residual glycogen content was lowered, the change was not significant (Table I).

Using sodium [3-^{14}C] pyruvate as substrate (Table II), hexobendine resulted in a significant reduction in pyruvate uptake and $^{14}CO_2$ production. A significant increase in pyruvate uptake, lactate and $^{14}CO_2$ production were observed when the hexobendine-treated hearts were driven at a constant increased rate (200 ± 3).

Hexobendine caused a significant decrease in palmitate uptake, $^{14}CO_2$ production and the percentage conversion of [^{14}C] palmitate to $^{14}CO_2$ (Table III). Pacing the hearts, after hexobendine administration, the metabolic pattern was reversed. Palmitate uptake, $^{14}CO_2$ production and percentage conversion of palmitate to $^{14}CO_2$ were significantly increased. With palmitate as substrate, coronary flow rate and oxygen uptake were depressed after hexobendine administration and increased when paced, but the changes were not significant.

TABLE I. THE EFFECT OF HEXOBENDINE ON THE METABOLISM OF [U^{14}C] GLUCOSE (10 mM) BY THE PERFUSED RAT HEART

	Heart rate	Glucose uptake	$^{14}CO_2$ production	Lactate production	Pyruvate production	[U-^{14}C] glucose incorporation into glycogen	Residual glycogen content
Control	**200**	**33·02**	**3·35**	**11·44**	**1·45**	**1.96**	**7.30**
(6)	**±3**	**±3·46**	**±0·63**	**±0·26**	**±0·19**	**±0·32**	**±1·26**
Hexobendine	**136**	**22·11**	**1·30**	**8·62**	**1·30**	**2.17**	**6.99**
(6)	**±6**	**±2·26**	**±0·22**	**±0·50**	**±0·23**	**±0·21**	**±0·46**
Hexobendine +	**200**	**20·45**	**±1.63**	**13·39**	**1·33**	**1.95**	**5·24**
pacing (6)	**±3**	**±1·84**	**±0·42**	**±0·60**	**±0·32**	**±0.32**	**±0.83**
P_1 <	**0·001**	**0·025**	**0·02**	**0·001**	**N.S.**	**N.S.**	**N.S.**
P_2 <	**0·001**	**N.S.**	**N.S.**	**0·001**	**N.S.**	**N.S.**	**N.S.**

Number in parentheses indicates number of hearts.
All results expressed as μmoles glucose eqv./g wet weight/30 min.
P_1 indicates significance of difference between control and hexobendine hearts.
P_2 indicates significance of difference between hexobendine and hexobendine + pacing.

TABLE II. THE EFFECT OF HEXOBENDINE ON THE METABOLISM OF [3-^{14}C] PYRUVATE (6 mM) BY THE PERFUSED RAT HEART

	Heart rate	Pyruvate uptake	Lactate production	$^{14}CO_2$ production	Residual glycogen content
Control	**202**	**57·67**	**23·48**	**15·69**	**12·69**
(6)	**±6**	**±4·00**	**±2·94**	**±0·29**	**±1·59**
Hexobendine	**130**	**45·99**	**19·30**	**10·20**	**14·21**
(6)	**±4**	**±1·90**	**±1·20**	**±0·15**	**±1·55**
Hexobendine +	**200**	**55·24**	**28·06**	**12·56**	**11·56**
pacing (6)	**±3**	**±2·79**	**±2·93**	**±0·36**	**±1·30**
P_1 <	**0·001**	**0·025**	**N.S.**	**0·001**	**N.S.**
P_2 <	**0·001**	**0·02**	**0·02**	**0·001**	**N.S.**

Number in parentheses indicates number of hearts.
Results expressed as μmoles/g wet weight/30 min.
P_1 indicates significance of difference between control and hexobendine.
P_2 indicates significance of difference between hexobendine and hexobendine + pacing.

TABLE III. THE EFFECT OF HEXOBENDINE ON THE METABOLISM OF [-^{14}C] PALMITATE (0·7 mM) BY THE PERFUSED RAT HEART

	Heart rate	Palmitate uptake (titration)	^{14}C palmitate uptake	$^{14}CO_2$ production	[^{14}C] palmitate incorporation into tissue lipids	% conversion of palmitate uptake to $^{14}CO_2$	% conversion of palmitate uptake to tissue lipids
Control	209	3·68	4·40	0·95	3·36	22·56	76·12
(6)	±6	±0·43	±0·31	±0·12	±0·15	±2·30	±4·20
Hexobendine	92	2·32	3·02	0·53	3·17	12·04	72·21
(6)	±5	±0·31	±0·22	±0·07	±0·13	±1·50	±1·96
Hexobendine + pacing (6)	198	3·88	4·22	0·91	3·08	21·68	70·00
	±3	±0·34	±0·23	±0·11	±0·16	±2·10	±2·60
P_1 <	0·001	0·05	0·05	0·01	N.S.	0·005	N.S.
P_2 <	0·001	0·01	0·01	0·02	N.S.	0·005	N.S.

Number in parentheses indicates number of hearts.
All results expressed as μmoles/g wet weight/30 min.
P_1 indicates significance of difference between control and hexobendine.
P_2 indicates significance of difference between hexobendine and hexobendine + pacing.

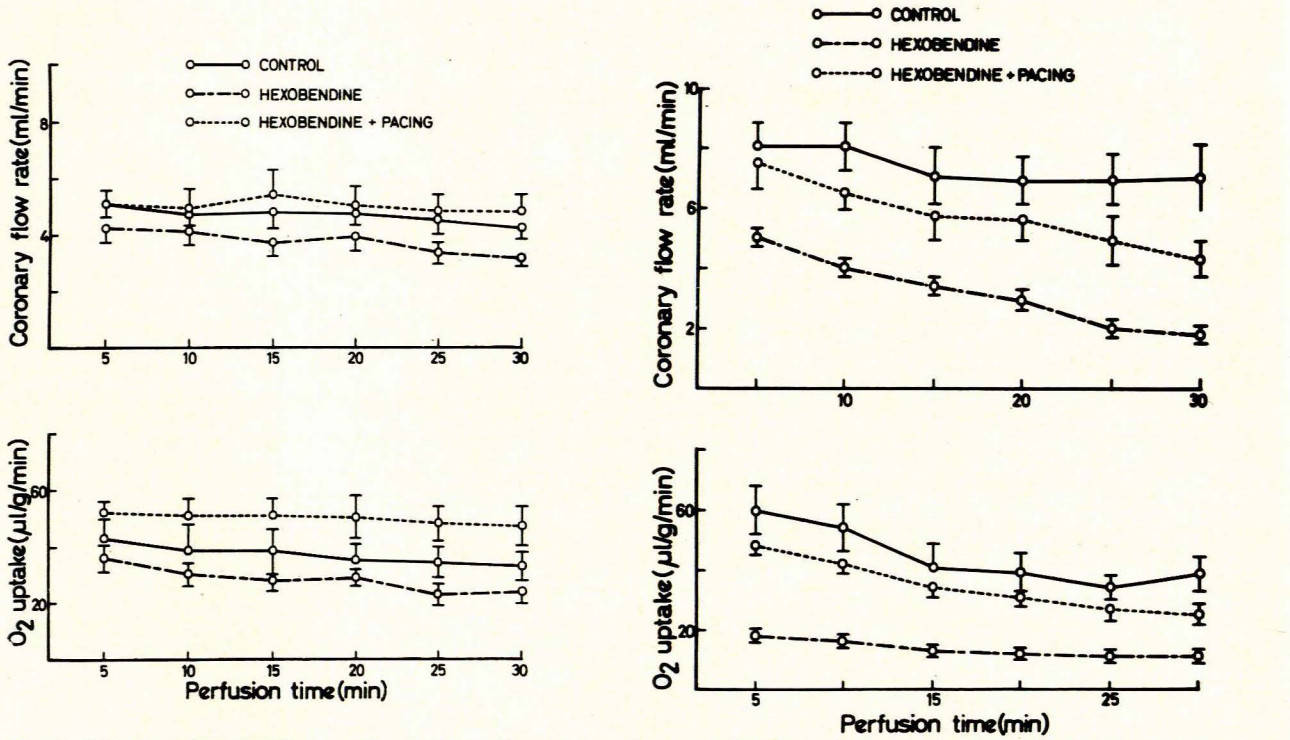

Fig. 1. Effect of hexobendine on O_2-uptake and coronary flow rate in the perfused rat heart. Left: **Substrate 10 mM glucose.** Right: **Substrate 0·7 mM palmitate.**

With glucose as substrate, hexobendine caused a significant reduction in coronary flow rate and oxygen uptake (Fig. 1). Pacing the hexobendine-treated hearts with glucose as substrate, resulted in a significant increase of oxygen uptake and coronary flow rate.

The high-energy phosphate contents were determined after a 30-min perfusion period, using 10 mM glucose as substrate (Table IV). ATP accumulated significantly after hexobendine addition, while no change was observed in the ADP, AMP, CrP and Pi contents.

Effect of Hexobendine on Mechanical Work Performance

The effect of hexobendine was studied in 3 series of rat hearts:

1. Control rat hearts were perfused over a period of 45 minutes.
2. Hearts were perfused without hexobendine for the first 15 minutes, serving as a double control, after which hexobendine was added and the effect on

TABLE IV. THE EFFECT OF HEXOBENDINE ON THE HIGH-ENERGY PHOSPHATE CONTENTS OF THE PERFUSED RAT HEART

	Heart rate	ATP	ADP	AMP	CrP	Pi
Control	**205**	**5·57**	**1·70**	**0·40**	**4·28**	**6·58**
(6)	**±6**	**±0·44**	**±0·20**	**±0·11**	**±0·21**	**±0·29**
(6)	**110**	**7·19**	**1·53**	**0·27**	**4·67**	**6·09**
Hexobendine	**±4**	**±0·40**	**±0·19**	**±0·09**	**±0·45**	**±0·43**
P <	**0·001**	**0·025**	**N.S.**	**N.S.**	**N.S.**	**N.S.**

Results expressed as μmoles/g wet weight/30 min.
Number in parentheses indicates number of hearts.
P values indicate significance of difference from control.

muscle mechanics observed over the next 30 minutes.

3. In order to eliminate the effect of reduced heart rate on the mechanical performance, a third series of hearts were perfused without hexobendine for the first 15 minutes, followed by hexobendine administration and stimulation with a pacemaker to maintain a control heart rate (200 ± 3).

These results are summarized in Fig. 2.

Hexobendine had a negative chronotropic effect as indicated by the significant reduction in heart rate (control 200 ± 3; hexobendine 139 ± 6). Tension development was depressed as indicated by the reduction in DT/dt_{MAX} and increase in t-PH. Stress relaxation was decreased. Tension time index (TTI) was elevated only for the first 15 minutes after hexobendine administration; thereafter it approached control values.

To eliminate the negative chronotropic effect of hexobendine, the hearts were driven at a constant control rate, using a pacemaker. Under these circumstances stress relaxation and DT/dt_{MAX} approached control values. Time to peak height of developed tension was reduced, while tension time index was elevated.

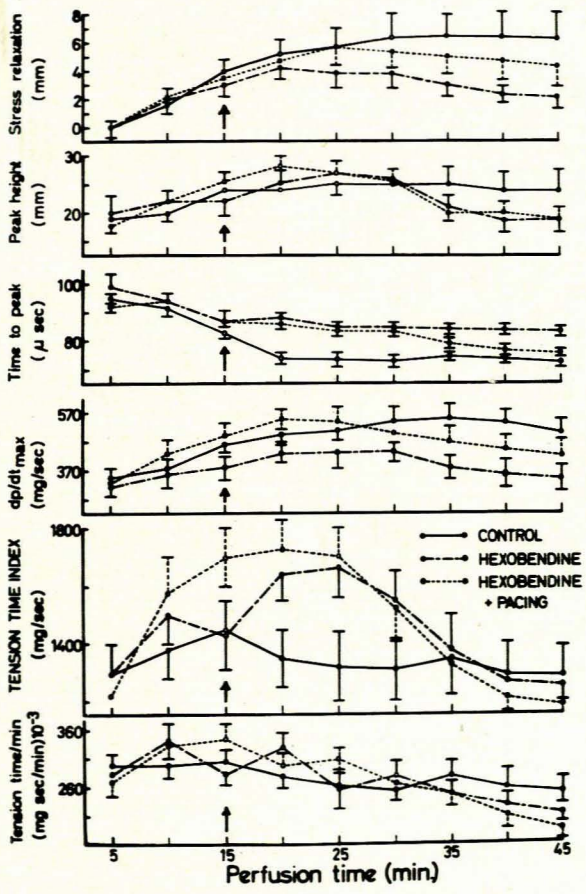

Fig. 2. Effect of hexobendine on the mechanical performance of the perfused rat heart.

DISCUSSION

The results obtained indicate that hexobendine resulted in a significant negative chronotropic effect in the isolated perfused heart. Due to this negative chronotropic effect of hexobendine, a significant reduction in substrate uptake and metabolism, as well as function, was observed. These results are not in accordance with the previous findings of Kraupp *et al.*[3,4] who found that hexobendine increased the rate of entry of glucose into the myocardium of the anaesthetized dogs, as well as an increased coronary blood flow rate.

In order to eliminate this negative chronotropic effect, the hearts were paced at a control rate (200 - 210 beats/min) and the subsequent metabolism and mechanical activity compared with those of spontaneously beating control hearts. Although it has been shown that field stimulation causes liberation of norepinephrine in isolated heart muscle,[34] which might influence myocardial metabolism, additional experiments performed in this laboratory showed that electrical pacing *per se* had no effect on the metabolism of control isolated perfused rat hearts. It was therefore decided to use spontaneously beating hearts as controls.

Pacing the hexobendine-treated hearts to eliminate the reduced heart rate, a significant increase in pyruvate and palmitate uptake and metabolism were observed. This indicates that the reduction in the uptake and metabolism of palmitate and pyruvate was due to the significant reduction in heart rate and not to the inhibition of these substrates on an enzymatic level by hexobendine. On the other hand, when pacing the hexobendine-treated hearts with glucose as substrate, no increase in uptake was observed. However, unpublished results from this laboratory have showed no increase in glucose uptake with increasing

heart rates. ATP accumulated significantly after hexobendine application, which might be attributed to the reduction in heart rate. A similar accumulation of cardiac ATP was observed by Kraupp *et al.*[15]

Although substrate uptake and metabolism were reduced after hexobendine administration, aerobic metabolism was not inhibited, since oxygen uptake was reduced not due to a specific inhibition of the Krebs cycle, but due to the negative chronotrophic effect of hexobendine, since the myocardial oxygen uptake increased when the hearts were paced (Fig. 1). Similarly, Kraupp *et al.*[15] observed no inhibition of aerobic metabolism in the rat heart after hexobendine injection.

The results further emphasized a significant negative inotropic effect, due to hexobendine. The findings of an increased t-PH and a reduction in DT/dt_{MAX} after hexobendine administration are evidence of an impairment in tension development. In contrast to the above findings, Kraupp *et al.*[3,4] observed increased heart work after hexobendine administration in anaesthetized dogs. The reason for this discrepancy is not clear, and further work is needed to elucidate the problem.

Increasing the heart rate by pacing the hexobendine-treated hearts caused stress relaxation and DT/dt_{MAX} to approach control values. Tension time index was also elevated by pacing. These findings suggest that the negative chronotropic effect of hexobendine is linked to the reduction in tension development.

The significant reduction in coronary flow rate after hexobendine administration, in contrast with the increased coronary blood flow in the anaesthetized dog[3,4] and in human patients,[16] may be due to dose differences or hormonal effects in the isolated rat heart. Results in this study showed no significant change in heart rate when using 4·5 μg hexobendine/ml perfusate, but at a concentration of 5·0 μg hexobendine/ml perfusate, a significant reduction in heart rate was obtained (Fig. 3).

In the light of the present study, it can be postulated that hexobendine relieves angina pectoris, as previously observed,[1] by virtue of its negative chronotropic effect whereby the oxygen consumption of the heart is reduced. These findings are not in accordance with the observations in the anaesthetized dog by Kraupp *et al.*,[3,4] who postulated that hexobendine, a coronary dilator, increased coronary blood flow with a decrease in oxygen consumption, thereby resulting in an increase in aerobic cardiac efficiency. Similarly, Brink and Lewis[16] observed in human patients an increase in coronary blood flow, but noted a decrease in pulse rate.

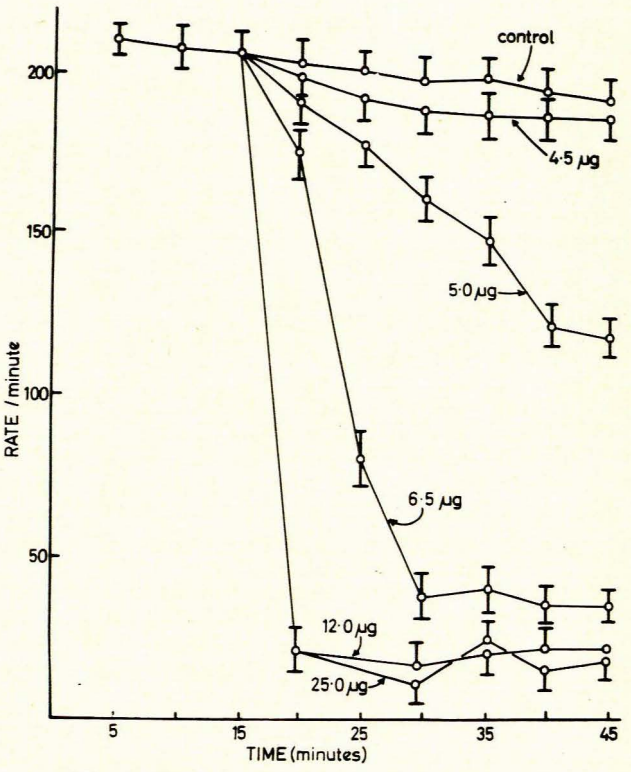

Fig. 3. Dose response curve of hexobendine.

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