ELECTROCARDIOGRAPHIC CHANGES PRODUCED IN RABBITS BY VASOPRESSIN (PITRESSIN) AND THEIR ALTERATION BY PROLONGED TREATMENT WITH A COMMERCIAL HEART EXTRACT

By J. W. Black*

From the Physiology Department, University of Glasgow Veterinary School, Glasgow, C.3

Received November 9, 1959

The electrocardiographic changes produced in rabbits by large doses of vasopressin are interpreted to arise from an initial, unstable, state of apical accelerated repolarisation followed by a more enduring state of subendocardial injury. When rabbits were treated, daily, with a commercial heart extract for two weeks and then given vasopressin two days after treatment had stopped, signs of apical accelerated repolarisation were unaltered but the signs of subendocardial injury were substantially suppressed.

There are several reports that a protein-free extract of heart muscle from freshly-killed young animals contains an unidentified principle which produces coronary dilatation in perfused, isolated, mammalian hearts[1–3] and improves the efficiency of contractions in heart-lung preparations[4] and in fatigued, isolated, cat papillary muscles[5]. Increased coronary blood flow[6] and antagonism to vasopressin-induced coronary vasoconstriction[7] have been observed in intact dogs. In all these preparations, the pharmacological effects of the extract are brief. However, the reports of clinical trials with the heart extract, in patients with coronary artery disease, attribute relatively long periods of improvement to the actions of the drug[8,9]. As clinical trials with new drugs in coronary artery disease are notoriously difficult to assess, it seeemed important to find out if this extract could produce any enduring changes in the pharmacological behaviour of the hearts of experimental animals. Lindner, Loudon and Werner[7] have shown that after three daily injections of the extract had been given to dogs, the electrocardiographic changes produced by vasopressin were reduced in comparison with the control period. However, two days after stopping the injections the cardiac sensitivity to vasopressin had returned to control levels.

This paper describes similar experiments in rabbits but with a longer period of treatment with the heart extract before the animals were challenged with vasopressin. As the electrocardiographic responses to vasopressin in rabbits were found to differ from those described for dogs[10,11,14], the effects of vasopressin in the untreated rabbits are given in detail in this paper.

* Present address: Research Department, I.C.I., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire.

METHODS

In all experiments, the animals were pretreated with either the heart extract or saline and then the cardiac responses to vasopressin were followed electrocardiographically. Rabbits were used for all experiments.

A water-soluble, protein-free, extract of heart muscle was supplied in ampoules. Each ampoule contained 1 ml. of extract equivalent to 0·97 g. of fresh heart, having between 100 to 115 mg. per cent total nitrogen content. A water-insoluble fraction was supplied in pills. Each pill contained 0·2 g. of dried material, equivalent to 0·82 g. of fresh heart, having between 11·3 to 12·3 g. per cent total nitrogen content. The treated groups of animals were given 1 ml. of water-soluble extract intramuscularly and one pill orally every day. The control groups of animals were given 1 ml. of sterile saline (0·9 per cent. w/v NaCl) intramuscularly every day but "dummy" pills were omitted. The animals were treated from Monday to Friday for two consecutive weeks. All control and treated animals were then given vasopressin (Parke-Davis, Pitressin, 20 i.u./ml.) on the Monday of the third week.

Electrocardiograms were taken with the animal, unrestrained, in the sitting position but movements were discouraged by surrounding the animal with a wooden barrier. A bipolar chest lead was used by inserting needle electrodes subcutaneously on both sides of the chest at the level of the 5th to 6th interspaces. The electrocardiograms were recorded with a New Electronic Products Multichannel Galvanometer Recorder. The overall frequency response of amplifier and galvanometer was flat to about 180 cycles/second. The amplifier time constant was 2 seconds and the sensitivity was adjusted to give 2 cm. deflection for 1 mV input.

Control electrocardiograms were taken over a period of 5 minutes and the vasopressin, in doses of 2 or 4 i.u./kg., was injected into an ear vein. Records were then taken at frequent intervals for at least 8 minutes.

Three experiments were carried out. In the *first experiment*, Himalayan doe rabbits about 9 months old were used. There were 6 control and 6 treated animals and at the end of the experiment all animals were given 2 i.u./kg. of vasopressin. The *second experiment* was a repetition of the first experiment but younger animals, about 3 to 4 months old, were used, and 4 i.u./kg. of vasopressin were given to each animal. To get satisfactory records from these animals they were sedated with 0·2 ml./kg. of 10 per cent w/v sodium thialbarbitone given intravenously. In the *third experiment*, 6 Dutch rabbits of similar age to those of group 1 (6 to 9 months old), were given 4 i.u./kg. of vasopressin and then the same animals were given the same dose of vasopressin after the standard period of treatment with the heart extract.

RESULTS

Effects of Vasopressin on the Electrocardiograms of Untreated Rabbits

The effects of vasopressin on heart rates, RS-T segments, and T waves are shown, for the three experiments, in Figures 1 and 2 and Table I.

Heart rates. After the injection of vasopressin, the rabbits went into

a shock-like state, usually with head-drop, and occasionally had to be prevented from falling over on their sides. The onset and recovery from this state of collapse seemed to be paralleled by cardiac slowing. The heart rate reached a minimum about 2 minutes after the injection and there was usually only moderate recovery after 8 minutes, when the recordings were stopped.

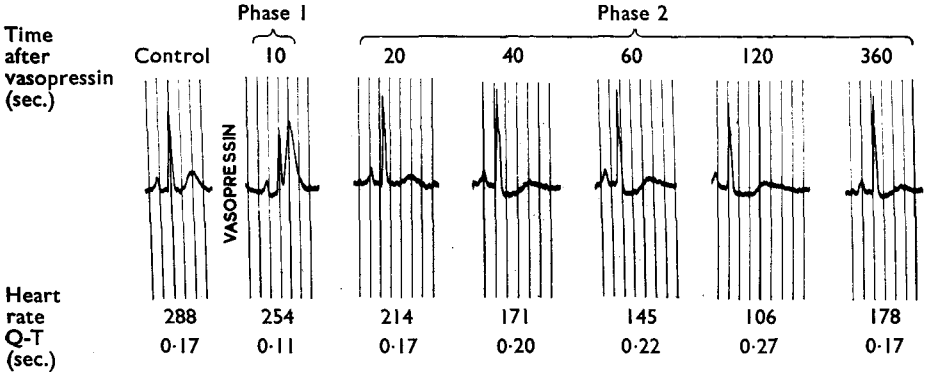


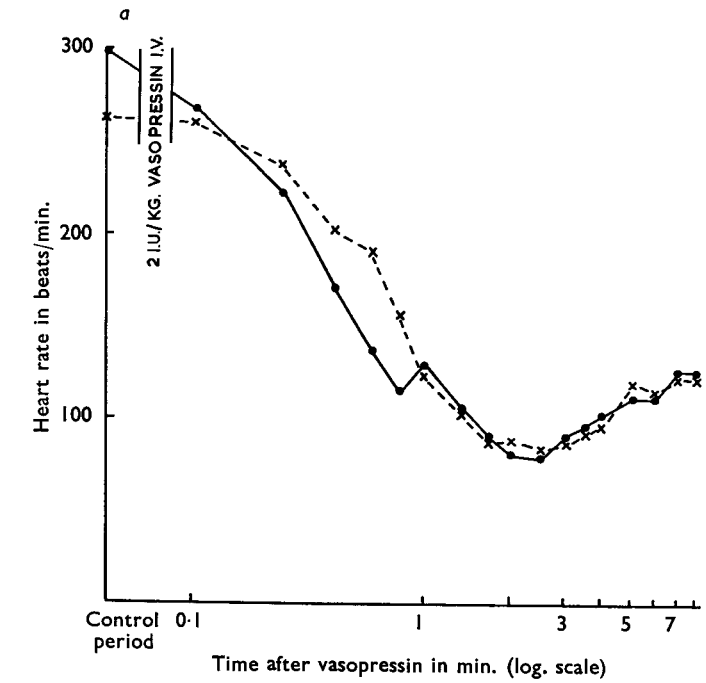
Fig. 1. Effects of vasopressin on the electrocardiogram of an untreated rabbit. Explanations of phases 1 and 2 are given in the text. Time marks = 0·04 sec.

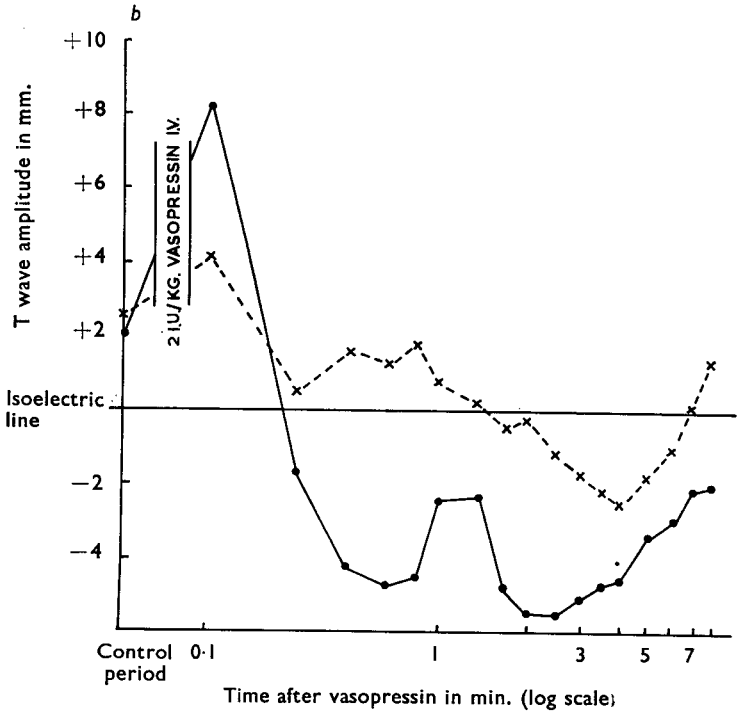
T waves. 30 control electrocardiograms were taken; 22 were found to have upright T waves, 3 had biphasic and 5 had inverted T waves.

After vasopressin, the first change was a marked elevation of T waves. The elevated T was accompanied by a shortened Q–T interval and hence high branching of the T wave from the downstroke of the R wave usually appeared (Fig. 1). These changes appeared within 5 to 10 seconds of the injection and had disappeared again within 25 to 30 seconds. After this phase, the T waves became depressed, and inverted T waves usually appeared within 1½ to 2 minutes of the injection and were slowly returning towards the control appearance after 8 minutes. This phase of depressed or inverted T waves was associated with long Q–T intervals. As the prolonged Q–T intervals occurred during the period of marked bradycardia, Hegglin and Holzmann's[12] empirical correction for frequency was applied to some of the results and showed that there was probably no significant relative increase of Q–T compared with the control values.

RS–T segments. With one minor exception, all electrocardiograms were found to have isoelectric RS–T segments during the control period. After the vasopressin injection there was a brief phase of elevated RS–T segments corresponding to the phase of elevated T and shortened Q–T intervals.

The RS–T segment usually became isoelectric again within 20 to 30 seconds after the injection. Between 30 to 60 seconds the RS–T segments became depressed, concave or flat, in all control experiments. In most experiments some RS–T segment depression was still present after 8 minutes. This phase of RS–T segment depression corresponded with


a
300
200
100
Heart rate in beats/min.
2 I.U./KG. VASOPRESSIN I.V.
Control period
0·1
1
3
5
7
Time after vasopressin in min. (log. scale)


b
+10
+8
+6
+4
+2
−2
−4
T wave amplitude in mm.
2 I.U./KG. VASOPRESSIN I.V.
Isoelectric line
Control period
0·1
1
3
5
7
Time after vasopressin in min. (log scale)

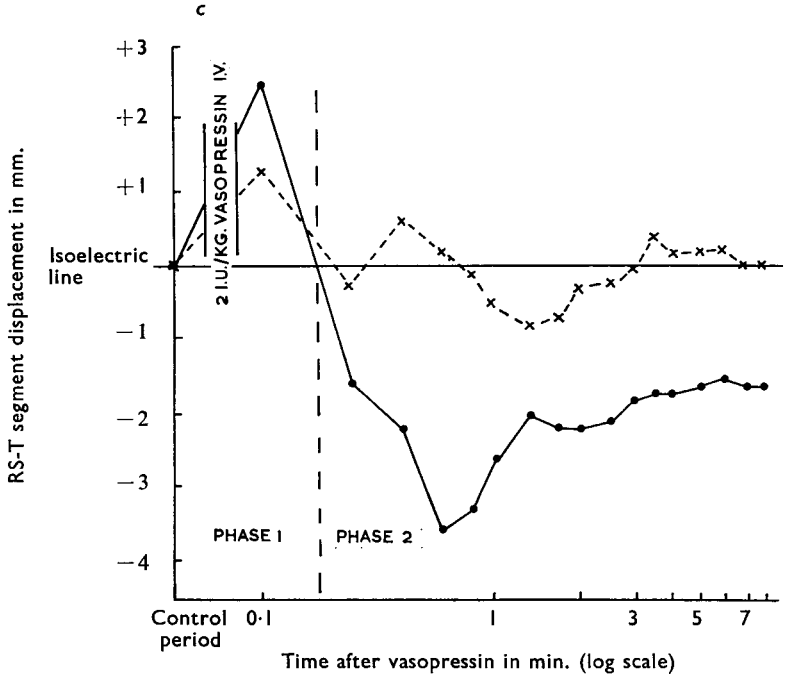


Fig. 2 *a,b,c.* Time-Course of electrocardiographic changes following vasopressin in untreated rabbits and in rabbits after treatment with heart extract.
The results are from Experiment 1 and each point plotted is the mean from each group of six animals. The standard errors of the means are included in Table I.

●——●, Untreated, ×– – –×, treated groups.

the phase of depressed or inverted T waves and normal or prolonged Q–T intervals.

On the basis of these changes in T, Q–T and RS–T the electrocardiograms of the control animals have been divided into two phases:

Phase 1 immediately follows the injection of vasopressin, lasts for 20 to 30 seconds, and is characterised by elevated T and RS–T and shortened Q–T intervals. *Phase* 2 succeeds phase 1, lasts for several minutes, and is characterised by depressed RS–T, depressed or inverted T, and relatively normal or prolonged Q–T intervals.

Effects of Pretreatment with Heart Extract on the Electrocardiograms after Vasopressin

Heart rates. In the three experiments there were no significant differences in heart rate responses to vasopressin between the treated and control groups of animals (Table I). By inference, it appears that pretreatment with the heart extract did not influence the generalised vasoconstriction produced by vasopressin.

T waves. There were no significant differences in T wave amplitude,

TABLE I

EFFECTS OF VASOPRESSIN ON HEART RATES, RS-T SEGMENT DISPLACEMENT AND T WAVE AMPLITUDE IN UNTREATED RABBITS AND RABBITS TREATED WITH HEART EXTRACT

Experiment No.	Time after vasopressin (sec.)	Heart rate: beats/min.			RS–T segment displacement: mm.			T wave amplitude: mm.		
		Untreated	Treated	P	Untreated	Treated	P	Untreated	Treated	P
1	Control	298 ± 8	272 ± 10	>0·1	0	0		+2·1 ± 0·92	+2·5 ± 1·25	>0·8
Phase 1	10	268 ± 17	260 ± 14	>0·7	+2·5 ± 1·70	+1·3 ± 0·80	>0·5	+8·3 ± 2·91	+4·1 ± 3·36	>0·3
Phase 2	40	171 ± 15	202 ± 21	>0·2	−3·6 ± 0·16	+0·2 ± 0·21	<0·01	−4·7 ± 1·06	+1·3 ± 1·27	<0·02
	120	81 ± 6	88 ± 15	>0·6	−2·2 ± 0·51	−0·3 ± 0·20	<0·05	−5·5 ± 1·12	−0·2 ± 1·15	>0·05
	360	127 ± 8	123 ± 7	>0·7	−1·6 ± 0·49	+0·2 ± 0·18	>0·02	−2·9 ± 1·08	+0·1 ± 1·21	<0·1
2	Control	291 ± 6	296 ± 5	>0·5	0	0		+1·6 ± 1·02	+2·5 ± 1·30	>0·6
Phase 1	10	261 ± 11	284 ± 7	>0·1	+2·3 ± 1·52	+1·6 ± 0·77	>0·6	+10·0 ± 2·58	+12·5 ± 2·86	>0·5
Phase 2	40	169 ± 17	207 ± 22	>0·2	−4·0 ± 0·73	+0·2 ± 0·85	<0·01	−2·9 ± 1·10	+2·8 ± 1·25	<0·02
	120	212 ± 9	147 ± 16	>0·2	−1·6 ± 0·48	−1·0 ± 0·36	>0·3	−2·7 ± 0·98	+0·6 ± 1·41	>0·1
	360	150 ± 11	160 ± 7	>0·4	−0·9 ± 0·28	−0·2 ± 0·19	<0·1	−2·0 ± 1·01	+2·4 ± 1·47	>0·05
3	Control	286 ± 11	285 ± 9	>0·9	0	0		+2·0 ± 0·57	+1·7 ± 0·85	>0·7
Phase 1	10	270 ± 12	288 ± 10	>0·3	+2·2 ± 0·61	+1·5 ± 0·73	>0·4	+12·6 ± 1·58	+7·2 ± 2·76	<0·2
Phase 2	40	177 ± 23	156 ± 21	>0·5	−1·4 ± 0·33	−0·1 ± 0·20	<0·02	+1·3 ± 0·25	+0·4 ± 0·60	>0·2
	120	134 ± 10	110 ± 7	>0·1	−3·6 ± 0·41	−0·9 ± 0·44	<0·01	−1·4 ± 0·47	+1·1 ± 0·69	<0·05
	360	146 ± 6	153 ± 5	>0·4	−0·4 ± 0·28	0		+0·6 ± 1·00	+0·3 ± 0·92	>0·8

Figures are the means and standard errors of the individual measurements.
P is the probability, estimated by *t* test, that the observed differences occurred by chance.

between treated and untreated animals, during phase 1 of the electrocardiograms. However, at the peak of phase 2, the T waves of the treated animals were significantly less depressed or inverted (Table I).

RS–T segments. There were no significant differences in RS–T segment elevation, between treated and untreated animals, during phase 1 of the electrocardiograms. At the peak of phase 2, however, the RS–T segments of the treated animals were significantly less depressed.

Discussion

Vasopressin produces coronary vasoconstriction and electrocardiographic signs of myocardial ischaemia[10]. Similar electrocardiographic signs do not appear when the cardiac muscle mass is uniformly ischaemic[13]. There is evidence, however, that the subendocardial region of the left ventricle is particularly susceptible to anoxaemia. Thus Lepeschkin[14] has emphasised that the subendocardial layers receive blood only during diastole while the subepicardial layers receive blood throughout the whole cardiac cycle. He believes that this explains why the subendocardial muscle layers are more sensitive than the rest of the heart to all types of coronary insufficiency. Dearing, Barnes and Essex[15] have shown that large doses of vasopressin produce cardiac necrosis which is most marked in the subendocardial region.

Using epicardial-facing electrodes, as in the present experiments, experimental subendocardial injury is known to produce RS–T segment depression, depressed or inverted T waves, and normal or prolonged Q–T intervals[14]. In the present experiments, similar changes were found during phase 2 of the electrocardiograms. This is the basis for believing that phase 2 is the result of subendocardial injury.

The significance of phase 1 of the electrocardiograms is more difficult to interpret. With epicardial-facing electrodes, elevation of RS–T and T imply subepicardial changes. The shortened Q–T intervals indicate that there has been a shortening of the recovery phase of the monophasic action potentials or accelerated repolarisation. Nahum and Hoff[16] have shown that warming the apex of the heart will produce similar electrocardiographic changes. Hence, phase 1 has been tentatively interpreted to arise from a brief period of accelerated repolarisation involving the whole apical muscle mass which appears before well marked injury has developed subendocardially.

In the present experiments, it was found that, in the groups of animals treated with heart extract, phase 2 of electrocardiographic changes after vasopressin was less marked than in the control animals, but that the phase 1 changes were not significantly different from the controls. If the interpretations of the two phases are substantially correct, then the heart extract treatment has apparently protected the hearts against subendocardial injury but has not influenced the initial phase of apical accelerated repolarisation.

This differential effect on the electrocardiogram and the persistence of the effect for at least two days after treatment had stopped, make it

difficult to explain the results in terms of coronary vasodilatation. However, Witzleb, Gollwitzer-Meier and Donat[4] have inferred from their experiments that the heart extract has a damping effect on the oxidative metabolism of the heart and increases the efficiency of myocardial contractions. This effect seems to be a possible explanation for the protection against subendocardial injury found in the present experiments. This would be an interesting explanation because Cossio[17] has recently suggested that iproniazid (marsilid) relieves ischaemic pain by depressing certain oxidative enzymes in the heart followed by an increased efficiency of oxygen utilisation.

It is usually difficult enough to assess the significance of experiments with tissue extracts said to contain unidentified active principles but more so in this case when the extract contains both water-soluble and water-insoluble components. Many experiments have been carried out with the water-soluble fraction but there appear to have been no pharmacological studies with the water-insoluble components. Both components were used, without prejudice, in the present experiments to mimic the clinical situation. Clearly, a separate pharmacological evaluation of each of these components is needed.

The results presented in this paper are consistent with previous reports about the activity of this heart extract, and taken together seem sufficiently interesting to warrant more experimental and clinical investigations on the nature of the active substances involved.

Acknowledgement. The heart muscle extract (Recosen) used in these experiments was made available by Dr. N. Levinson of Robapharm Ltd., Basle, Switzerland, to whom I am also indebted for supplying much useful information about this preparation.

REFERENCES

1. Haemmerli, *Helv. med. acta*, 1952, **17**, 9.
2. Ryser and Wildbrandt, *Arch. int. Pharmacodyn.*, 1953, **96**, 131.
3. Stern, *Z. Kreis. Forsch.*, 1951, **40**, 1726.
4. Witzleb, Gollwitzer-Meier and Donat, *Klin. Wschr.*, 1954, **32**, 297.
5. Loubatieres and Sassine, *Arch. int. Pharmcodyn.*, 1953, **95**, 246.
6. Blömer and Schimert, *Schweiz. med. Wschr.*, 1951, **81**, 1108.
7. Lindner, Loudon and Werner, *ibid.*, 1923, **83**, 360.
8. Weiss, *ibid.*, 1952, **82**, 767.
9. Greif and Höfler, *Wein. med. Wschr.*, 1951, **101**, 850.
10. Melville, *J. Pharmacol.*, 1938, **64**, 86.
11. Gruber and Kountz, *Proc. Soc. exp. Biol., N.Y.*, 1929–30, **27**, 161.
12. Hegglin and Holzmann, *Z. klin. Med.*, 1937, **132**, 1.
13. Brofman, Leighninger and Beck, *Circulation*, 1956, **13**, 161.
14. Lepeschkin, *Modern Electrocardiography*, Vol. I, Bailliere, Tindall and Cox, London, 1951, S, 797, 831.
15. Dearing, Barnes and Essex, *Amer. Heart J.*, 1944, **27**, 96.
16. Nahum and Hoff, *Amer. J. Physiol.*, 1948, **155**, 215.
17. Cossio, *Amer. Heart J.*, 1958, **56**, 113.