THE EFFECTS OF PROTOVERATRINE ON PLASMA POTASSIUM LEVELS IN THE CAT AND RABBIT

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Protoveratrine has been shown to increase the level of venous plasma potassium (K^+) in intact rabbits and anaesthetised cats. By the use of restricted circulation experiments it has been demonstrated that at therapeutic dose levels protoveratrine causes an increase in the level of K^+ in the plasma of blood from the coronary circulation but not from skeletal muscle. The possible significance of this finding and its relation to the Bezold-Jarisch reflex is discussed.

PURIFIED preparations of the veratrum alkaloids have been used to reduce blood pressure in hypertension without the production of ganglion blockade[1]. The alkaloids act mainly on peripheral sensory receptors[2] and appear to make them more sensitive to their normal stimulus[3,4,5]. In 1939 Bacq[6] showed that veratrine could sensitize frog muscle to the stimulant action of the potassium ion (K^+), and more recent work has shown that this property is also possessed by the pure ester alkaloids[7].

We have made a number of experiments with the purified ester alkaloid protoveratrine to find if the intravenous injection of this alkaloid produces an increase in the K^+ concentration of the blood plasma. Samples of blood were taken from different vascular beds and the plasma K^+ concentration determined.

MATERIALS AND METHODS

The protoveratrine was a mixture of the hydrochlorides of protoveratrine A and protoveratine B in the proportions of 2:1. It was obtained as a solution containing 0·1 mg./ml. of alkaloids in a 0·6 per cent w/v solution of sodium chloride. The control solutions consisted of 0·9 per cent w/v sodium chloride.

Plasma K^+ of Rabbit Venous Blood

Rabbits of either sex weighing from 2·25 to 5·0 kg. were used. The animals were gently restrained and two 1 ml. samples of blood taken from one marginal ear vein. The appropriate volume of drug solution was adjusted to 1 ml. with 0·9 per cent w/v sodium chloride and injected into the marginal vein of the other ear 10 minutes after taking the first blood samples. Further samples were taken from this ear 2 and 20 minutes after the drug injection.

The blood was collected into graduated centrifuge tubes each containing 100 i.u. of heparin. The heparinised blood was centrifuged in a M.S.E. angle head centrifuge at 3600 r.p.m. for 10 minutes. Three 0·2 ml. samples of plasma were pipetted from each tube, diluted with K^+-free distilled water and the K^+ concentration determined with an E.E.L.

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flame photometer and expressed as m-equiv./litre. Samples showing haemolysis were discarded.

Plasma K^+ Level of Blood from the Cardiopulmonary Region of Spinal Cats

The spinal cords of cats were cut in the cervical region under ether anaesthesia and the brains destroyed. Ninety minutes were allowed to elapse to allow for elimination of the anaesthetic and for ionic equilibration following surgical trauma.

Drugs were injected into a cannula in the external jugular vein and blood samples taken from a siliconed glass cannula in the left common carotid artery. Two 1 ml. blood samples were taken for control purposes before the drug injection and then at 2 and 30 minutes after the injection, because the hypotension produced by intravenous protoveratrine in lightly anaesthetized cats reaches its maximum at 2 minutes and disappears after 30 minutes. Blood samples were collected and plasma K^+ concentrations estimated as described above.

Doses of 10 or 20 μg. of protoveratrine were given and the number of doses administered to each animal limited to three to prevent the development of tachyphylaxis[2].

K^+-Release from Cat Skeletal Muscle

The effects of protoveratrine on the release of K^+ from skeletal muscle were studied in the denervated hind limbs of pentobarbitone-anaesthetised cats. One limb was used to test the effects of the drug and the contralateral limb as a control.

Intra-arterial injections were made into a needle type injection cannula tied into a minor branch of the femoral artery high in the thigh in such a way that the tip of the cannula was at the junction of this branch with the femoral artery. A similar cannula was tied into a branch of the femoral vein at the same level in the limb.

Total doses of 2 or 5 μg. of protoveratrine in a volume of 0·1 ml. were injected into the femoral artery and blood samples of 0·5 ml. removed from the vein 5, 10, 30, 60 and 120 seconds after injection. Simultaneous injection of the control solution into the contralateral leg and collection of blood samples were made for the purpose of comparison.

Arterial blood pressure was recorded from the left common carotid artery by means of a mercury manometer.

K^+-Release from the Heart of Anaesthetised Cats

As a large component of the reflex hypotensive effect of protoveratrine is believed to arise from receptors in the coronary bed[3,5] the effect of injection of small doses of protoveratrine into the left common coronary artery on the K^+ concentration of coronary venous blood plasma was studied.

The technique used was a modification of that described by Dawes[8] for the administration of drugs into the left common coronary artery combined with the technique first described by Morawitz and Zahn[9] for obtaining samples of blood from the coronary sinus.

Cats of either sex weighing from 3·5 to 6·0 kg. were used. They were anaesthetised by intraperitoneal injection of 40 mg./kg. of sodium pentobarbitone. The thorax was opened on the left side between the fourth and fifth ribs and the animal artificially ventilated using a Starling respiration pump. All bleeding points were sealed with electro-cautery. The space between the ribs was enlarged using rib retractors and the heart and great vessels exposed. The left subclavian artery was cleared of connective tissue and the pericardium incised and reflected. The left

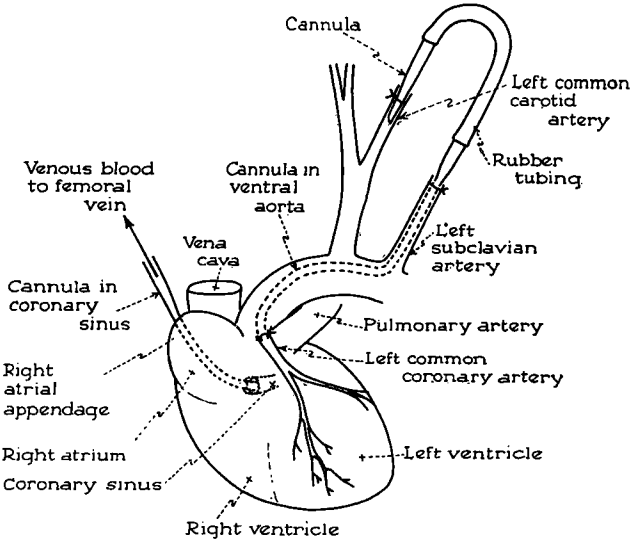

Fig. 1. *Position of cannulae in cat heart.*

common coronary artery was then cleared from adhering fatty tissue and a loose ligature passed round it. The coronary sinus cannula was introduced into the right atrium through an incision in the right atrial appendage, the tip introduced into the coronary sinus and the cannula secured by means of a drawstring suture round the incision in the appendage. A siliconed glass cannula similar to that described by Dawes[8] was introduced into the left subclavian artery and, guided by touch, pushed caudally until its tip was in the region of the lumen of the left common coronary artery. Its distal end was connected by a piece of flexible rubber tubing to a glass venous type cannula in the left common carotid artery. The tip of the cannula was tied into the left coronary artery thus establishing a closed circuit (Fig. 1).

Blood pressure was recorded from the right femoral artery and a continuous E.C.G. recording was taken from lead II using subcutaneously implanted needle electrodes. The record was made and displayed with an Ediswan direct writing pen recorder. After the operation was complete the animal was heparinised and given an intravenous infusion of 15 ml. of normal saline to restore the blood volume. Drugs were introduced

TABLE I

THE EFFECTS OF PROTOVERATRINE ON THE PLASMA K^+ OF INTACT RABBITS

Experiment No.	Sex	Age in months	Wt. in kg.	Dose μg./kg.	Mean K^+ levels of plasma m-equiv./l.			Symptoms
					Before drug	After drug		
						2 min.	20 min.	
1	M	*c.* 24	5	10	4·43	3·76	3·74	No obvious signs of discomfort. Some bradycardia
2	M	5	2·5	10	4·98	5·50	4·95	ditto
3	M	6	2·8	10	3·31	3·83	4·47	ditto
4	F	5	2·8	10	4·46	4·98	4·60	ditto
5	M	5	2·75	20	5·63	5·67	5·63	Slight signs of discomfort, retching
6	M	6	4·5	20	4·60	5·88	4·75	Sneezing, loss of muscular tone, tachypnea, bradycardia
7	M	5	3·1	20	5·10	5·26	5·36	ditto
8	M	3	2·75	20	3·58	5·88	4·60	Sneezing, muscular tremors, attempted vomiting, periods of apnea, bradycardia
9	M	6	2·25	20	3·66	5·26	4·60	Sneezing, retching, salivation, bradycardia
10	F	6	3·0	20	4·56	5·45	5·05	ditto
11	M	6	2·25	30	4·34	4·60	4·35	Attempted vomiting, irregular respiration and apnea, slight convulsions, bradycardia
12	F	3	2·0	30	4·75	16·8	23·1	Spasmodic head movements, attempted vomiting, extension of neck, followed by loss of muscular tone, convulsions and death

TABLE II

THE EFFECT OF PROTOVERATRINE ON THE PLASMA K^+ LEVEL OF CAROTID BLOOD IN SPINAL CATS

			Sampling time in minutes																		
Experiment No.	Sex	Weight in kg.	90	120		150		180		210		240		270	300	330		360		390
1	F	2·5				4·35		4·25	*4·87*	5·33										
2	F	3·5	3·58	4·35						3·84	*3·80*	4·20			6·30	8·40				
3	M	2·75	4·1	5·30				5·30				6·40								
4	F	2·5	4·6	4·25		5·45				4·75	*4·82*	5·20		5·20	6·90	6·15	*6·65*	7·30		
5	F	2·6	4·95	4·75		5·20				5·10	*5·21*	5·37		5·10						
6	F	3·0	2·76			3·50				4·20				3·40	4·20	5·0	*5·75*	3·9	4·3	4·6
7	M	3·25	2·98	3·23				3·33		3·33	3·92	3·94		3·46	3·90	4·8	5·24	4·08		
8	F	3·25	3·40	3·71				3·96		3·71	*3·35*			3·56	4·10	3·46	3·86	3·22	3·76	3·96
9	F	3·2	2·80	2·70	2·80	2·78		3·32		3·20	5·4	4·6		6·1						
10	F	2·0	3·54	3·68		3·42	4·8	3·68												
11	M	2·6	2·62	3·08				3·76		4·23		5·0	5·0	5·0	4·25					

Italic figures = K^+ level 1 minute after the injection of 10 μg. protoveratrine.
Bold figures = K^+ level 1 minute after the injection of 20 μg. protoveratrine.
All K^+ concentrations expressed as m-equiv. of K^+ per litre of plasma.

into the coronary circulation by injection through the rubber tubing joining the two cannulae. Blood drained from the coronary sinus was reintroduced into the right femoral vein.

Doses of 2 or 5 μg. of protoveratrine in 0·1 ml. of solvent or the equivalent volume of control solution were introduced into the coronary circulation. The effects of the drug on the blood pressure and E.C.G. were recorded. The doses of drugs used, produced a fall in blood pressure and a bradycardia readily detected by the E.C.G. Control solutions produced only a transient fall in blood pressure and no detectable bradycardia.

The maximum changes in blood pressure and heart rate occurred from 2 to 5 seconds after injection of the drug and at these times samples of coronary venous blood were taken and analysed for plasma K^+ using the method previously described.

Results

Plasma K^+ Levels in Rabbit Venous Blood

The object of this series of experiments was to determine if any gross changes in plasma K^+ levels occurred after intravenous injection of protoveratrine in doses sufficient to produce a marked fall in blood pressure.

Fairly high doses of protoveratrine (10, 20 or 30 μg./kg.) were used to produce a very marked effect. At the 10 μg./kg. level no obvious toxic symptoms were observed but bradycardia was noticed in two out of the four animals used. With higher doses signs of distress became apparent. The plasma K^+ levels before and after protoveratrine are shown in Table I. Plasma K^+ levels before injection of the drug were within the range given by Spector[10], *i.e.* 2·7 to 5·1 m-equiv./litre, in eleven of the twelve experiments. In these the plasma K^+ level was significantly raised after intravenous injection of protoveratrine ($P = 0{\cdot}05$). The rabbit in experiment No. 12 died showing a high plasma K^+ level before death. This animal was not included in the statistical test for significance. At the lowest doses, the increase in K^+ levels was less marked but occurred in three out of the four experiments.

Plasma K^+ Levels of Blood from the Cardio-Pulmonary Circulation

The K^+ concentration of arterial plasma showed marked variations between experiments but was within the range reported by Cattel and Civin[11]. As the experiment proceeded, the concentration rose. This was expected, as operative trauma has been shown by a number of workers to produce an increase in plasma K^+[11–13].

The injection of protoveratrine into the jugular vein frequently gave rise to bradycardia presumably owing to its direct negative chronotropic effect on the heart[2]. The results from eleven experiments are summarised in Table II.

After the injection of 10 μg. protoveratrine the K^+ level rose in six out of eight and with 20 μg. in seven out of eight experiments. No increase was observed after the injection of 0·2 ml. of control solution.

TABLE III

THE EFFECT OF PROTOVERATRINE ON THE K^+ LEVEL OF VENOUS PLASMA TAKEN FROM THE HIND LIMBS OF CATS

Experiment No.	Test: K^+ level of plasma m-equiv./l.						Control: K^+ level of plasma m-equiv./l.					
	Before drug	No. of seconds after drug					Before control	No. of seconds after control				
		5	10	30	60	120		5	10	30	60	120
For 2 μg. Protoveratrine												
1	4·21	4·23	4·20	4·26	4·18	4·21	4·19	4·22	4·17	4·22	4·20	4·18
2	3·76	3·74	3·80	3·71	3·72	3·78	3·80	3·81	3·78	3·92	3·73	3·87
3	3·98	4·10	3·89	4·00	4·01	4·00	3·96	3·96	4·00	3·89	3·94	3·96
4	3·41	3·36	3·41	3·40	3·39	3·50	3·40	3·41	3·37	3·61	3·54	3·44
	Mean ± SE 3·84 ± 0·31						Mean ± SE 3·86 ± 0·27					
For 5 μg. Protoveratrine												
1	4·23	4·24	4·19	4·26	4·20	4·20	4·21	4·22	4·19	4·29	4·18	4·20
2	3·66	3·68	3·57	3·65	3·70	3·69	3·80	3·81	3·69	3·84	3·75	3·72
3	4·12	4·12	4·20	4·15	4·08	4·10	4·00	4·20	4·22	4·16	4·06	4·04
4	3·50	3·41	3·40	3·57	3·49	3·52	3·51	3·41	3·42	3·61	3·44	3·49
	Mean ± SE 3·87 ± 0·32						Mean ± SE 3·89 ± 0·38					

The results indicate that injection of protoveratrine into the cardio-pulmonary region produced an increase in the plasma K^+ concentration. This rise may have been caused by the actual liberation of K^+ or to the drug-induced bradycardia reducing the rate of outflow from the coronary system, thus allowing more K^+ to accumulate in the venous outflow. The K^+ may also have originated in the heart or the lungs.

K^+ from Cat Skeletal Muscle

Intra-arterial injection of protoveratrine in total doses of 2 or 5 μg. produced no muscular twitching and in only two cats out of eight was hypotension produced by the drug and when this did occur it was only transient.

TABLE IV

THE EFFECTS OF PROVERATRINE ON THE K^+ CONCONCENTRATION OF BLOOD TAKEN FROM THE CORONARY SINUS

Experiment No.	Total dose of protoveratrine	K^+ concn. in m-equiv./l.		Per cent change
		Control	Drug	
1	2 μg. 2 μg. 5 μg.	1·73 1·73 4·02	1·90 1·78 5·30	+ 9·8 + 2·9 + 32·8
2	5 μg.	1·54	2·19	+ 35·7
3	2 μg. 5 μg.	4·86 4·09	5·11 5·37	+ 15·8 + 30·8
4	2 μg.	3·96	4·60	+ 16·2
5	2 μg.	2·94	3·58	+ 21·7
6	5 μg.	1·20	1·65	+ 37·5

Mean per cent increase in K^+ concentration after 2 μg. protoveratrine = 13·3 ± 3·2 per cent.
Mean per cent increase in K^+ concentration after 5 μg. protoveratrine = 34·2 ± 3·3 per cent.

The results of eight experiments are summarised in Table III. A statistical comparison of the means for the control and the drug-treated limbs showed that there was no significant difference ($P > 0·9$) between the plasma K^+ concentrations of the venous blood from the two.

K^+-Levels in Coronary Sinus Blood

Because of the technical difficulties involved in this series of experiments the mortality rate was high. In all the successfully executed experiments, the results from which are listed in Table IV, intra-coronary injection of small doses of protoveratrine led to a marked increase in K^+ concentration of the blood collected from the coronary sinus. The onset of bradycardia was taken to indicate that the drug had reached the receptor sites.

No increase in the K^+ concentration of coronary sinus plasma occurred after injection of 0·2 ml. of control solution into the coronary circulation.

It therefore appears that under the conditions of these experiments protoveratrine is capable of increasing K^+ efflux from heart muscle.

Discussion

Protoveratrine is a mixture of two very closely related pharmacologically active steroidal ester alkaloids which are qualitatively identical and differ chemically only in their acid moieties[14].

In addition to the action of protoveratrine on the cardiovascular system, as exemplified by the initiation of the Bezold-Jarisch reflex, it can alter the shape and size of the resting and action potentials of nerve and muscle and sensitize excitable tissue to the stimulant actions of K^+ [2,4].

It has been suggested by various authors that the veratrum alkaloids may affect the ionic balance of nerve and muscle cells causing an increase in extracellular K^+ levels[15,16]. Shanes[15] has demonstrated a greater K^+ loss from frog nerves exposed to veratrine than from the control nerve and using protoveratrine we have demonstrated that this drug can produce an increase in the plasma K^+ levels in intact rabbits and anaesthetized cats. Kahn and Acheson[17] have shown that this effect is not due to the release of K^+ from erythrocytes and as the greatest reservoir of K^+ in the body is the skeletal musculature this was considered a possible source of the increased plasma K^+. Our results show, however, that it is unlikely that this excess K^+ originates from skeletal muscle and that a more likely source appears to be the myocardium.

The amount of K^+ released is very small and we have had to use doses of protoveratrine higher than those used in therapy before any measurable change could be detected in the experiments upon the general circulation. Smaller doses of drug, within the range achieved in therapeutics, have been shown to cause an apparent release of K^+ into the restricted circulation of the heart but not from the skeletal musculature. Vick and Kahn[18] have recently demonstrated a similar increase in K^+ efflux from isolated perfused guinea pig hearts and our results appear to confirm their findings.

It is significant that, of the various types of sensory nerve endings which may be stimulated by protoveratrine, those which appear to be stimulated by the lowest concentration of drug, are situated within the walls of the heart in the area of distribution of the left coronary artery and can initiate the Bezold-Jarisch reflex[3,8]. It is possible, therefore, that the relatively high efflux of K^+ occurring in this region after protoveratrine may preferentially affect these endings and so facilitate initiation of this reflex.

Many factors are known to cause, or to be involved in, the release of intracellular K^+ and this study does not indicate the mechanism by which protoveratrine acts. Work on isolated organs and tissues using more refined techniques is reported elsewhere[19].

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After Dr. Lister presented both papers there was a DISCUSSION.