THE RESPONSE OF THE HEART TO VISAMMIN AND TO KHELLININ

By Karam Samaan, Ahmed M. Hossein and Ibrahim Fahim

From the Materia Medica Department, Kasr El Aini Faculty of Medicine, Cairo

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Introduction

It has already been reported that various active principles are present in the fruit of *Ammi Visnaga* Linn, with different pharmacological actions and therapeutic idications[1,2,3]. At the time of isolation of these principles in 1930 in this Department, the literature found on the chemistry of this drug was by Ibrahim Mostapha[4] and T. Malosse[5]. The latter described three crystalline principles which he named visnagine α, visnagine β, and visnagine γ. Ibrahim Mostapha described a crystalline principle which he named khellin, and which was stated by Malosse (*loc. cit.*) not to be identical with visnagine α, but was supposed to be formed during the process of extraction. In view of this and other anomalies (variation of m.pt., etc.) it was deemed preferable to give new names to the isolated compounds, names which are derived from the Latin and Arabic names of the drug—Ammi Visnaga and Khella respectively.

Two compounds of the various principles isolated, namely visammin and the glycoside khellinin, are of great interest pharmacologically.

Anrep *et al.*[6,7,8], who recently worked on the active principles of Ammi Visnaga, refer to khellinin as khellol glycosides regardless of the fact that khellinin is not an alcohol, and that names of glycosides end in " in." The same authors refer to visammin as khellin.

The value of Ammi Visnaga as an antispasmodic and as a coronary dilator was indicated by one of us as early as 1930[9]. In what concerns the action of visammin and of khellinin on the circulation, experimental animals being the toad, rabbit and dog, the following points were indicated. (a) Visammin[1] in a concentration of 1 : 100,000 diminishes the amplitude of beat and slows the heart with no arrest. Stronger solutions (1 : 50,000 to 1 : 20,000) produce more evident slowing and a great diminution of the amplitude of beat. The diminished amplitude is relatively much more marked than the slowing, and is, to a great extent, due to diminution of systole rather than of diastole. Atropine added to the perfusion fluid produces no evident changes in the amplitude. The action is considered to be a direct depressant effect on the muscle fibres. Visammin is a systemic and coronary vasodilator. The intravenous injection of visammin produces an immediate fall of blood-pressure which returns to normal. Later it falls slightly below the normal and remains so for a long time. The first fall of blood-pressure occurs before and after atropine, but is more marked when no atropine is injected before injecting the drug. Similarly the fall of blood-pressure is more marked when the vagi are intact than when severed. Pre-

sumably, therefore, the initial fall of blood-pressure is mainly cardiac in origin and is due to the direct action of visammin on cardiac muscle and to cardiac inhibition through stimulation of the vagal centre. The intestinal volume shows an early slight decrease which may be explained as a secondary effect to the immediate action of the drug on the heart. Soon, the splanchnic vessels dilate as evidenced by the increase in intestinal volume. The vasomotor centre appears to be stimulated as indicated by the return of blood-pressure to normal after the first fall with a secondary diminution in intestinal volume, while, lastly, the direct action of visammin on the muscle wall of the blood vessels overshadows the stimulant effect on the vasomotor centre and results in a final slight fall of blood-pressure with increase in intestinal volume. (b) Khellinin[1,3] in a concentration of 1 : 100,000 increases the contractility of the cardiac muscle by producing a more complete systole and a more complete diastole with a corresponding increase in cardiac output. It increases the coronary flow, and this increase is more pronounced if the coronary vessels were first rendered in a state of partial spasm by barium chloride (1 : 40,000). The intravenous injection of the glycoside with the vagi intact slightly raises the blood-pressure with an increase in intestinal volume. The rise in blood-pressure is cardiac in origin.

Recently Anrep *et al.*[6,7,8] and Kenawy and Barsoum[10,11] state that visammin is not a cardiac depressant, and that the administration in a heart-lung preparation of a dog of 100 mg. of the drug produces no change in the heart volume. The same authors record that the glycoside khellinin is not a cardiac stimulant and does not increase the coronary flow, and is devoid completely of pharmacological activity. Nevertheless, Bagouri[12], just recently, has admitted in his experiments that visammin causes a diminution in the amplitude of the heart beat which is of a temporary nature, and that khellinin produces a slight increase in the strength of heart beat with large doses of the glycoside.

This being the case, we thought it worth while to record the following experimental results and observations, in view of the fact that we have examined both principles in their crude forms and in their various stages of purity obtained during the processes of their final isolation—results and observations which may throw light on these variations of results.

EXPERIMENTAL

(A) *Intact animal:* The dog was used in all experiments. Anæsthesia was maintained by the intravenous injection of 0·22 g. of barbitone sodium per kg. supplemented by ether for the preliminary operative procedure. Blood-pressure was measured from the femoral artery. Injections were made in the femoral vein of the opposite side. In those experiments in which the ventricular beats were recorded, uniform artificial respiration was maintained by the use of Brodie's ideal respiration pump. The thorax was opened in the usual way. The pericardium was snipped through and ligatured to the anterior thoracic wall on each side.

A fine hook was applied to the tip of the left ventricle and connected to a recording lever. In some experiments the vagi were severed. We restricted ourselves to the injection of limited doses of visammin and of khellinin so as not to interfere with blood volume—the solubility of these principles in Ringer's solution being, indeed, very limited—and we avoided the interference of solvents (alcohol or sodium benzoate for visammin and alcohol or pyridine for khellinin) and the use of controls.

The injection of 3 to 4 mg. of pure visammin (m.pt. 153° to 154°C.) per kg. reduced the contractility of the ventricular muscle, producing principally a less complete systole. The diminution of the amplitude of beat was always evident, but was more marked when the vagi were intact (Fig. 1), and slowing of the rate of beat may later occur. The first

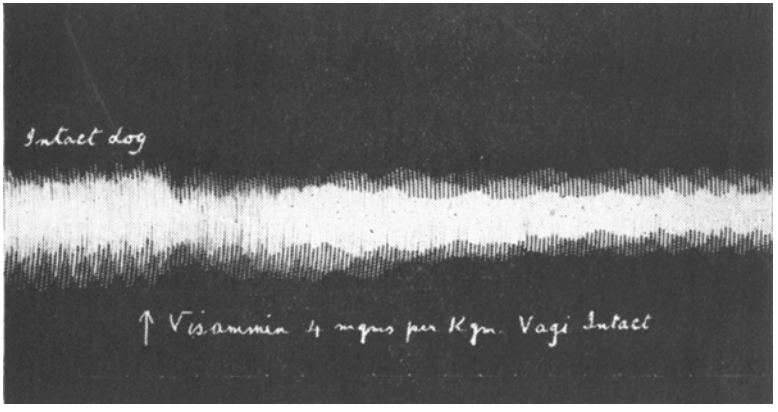

FIG. 1 (reduced).—Intact dog under barbitone sodium and uniform artificial respiration. The ventricular heart beats are recorded with the vagi intact. Intravenous injection of 4 mg. of visammin per kg. reduced the contractivity of the ventricular muscle producing principally a less complete systole.

effect on blood-pressure was an initial fall followed by recovery with fluctuation—as previously reported the direct depressant action of visammin on the heart and the vasodilatation by its direct action on the muscle wall of the blood vessels contribute to the lowering of blood-pressure, whereas the stimulant action of the drug on the vasomotor centre may overshadow this effect.

On the other hand, the injection of 1 to 1·5 mg. of pure khellinin (m.pt. 175° to 176°C.) per kg, increased the contractility of the ventricular muscle producing a more complete systole and a more complete diastole. The increase in amplitude of the ventricular beats was always evident, but was more marked when the vagi were severed (Fig. 2), the rate of beat hardly changed though, relatively, slowing of the heart beat may occur with the vagi intact. The glycoside in similar doses raised the blood pressure in the intact animal especially when the vagi were severed (Fig. 3). This effect is, however, in favour of increased cardiac output—the glycoside does not directly stimulate the vasomotor centre and has no

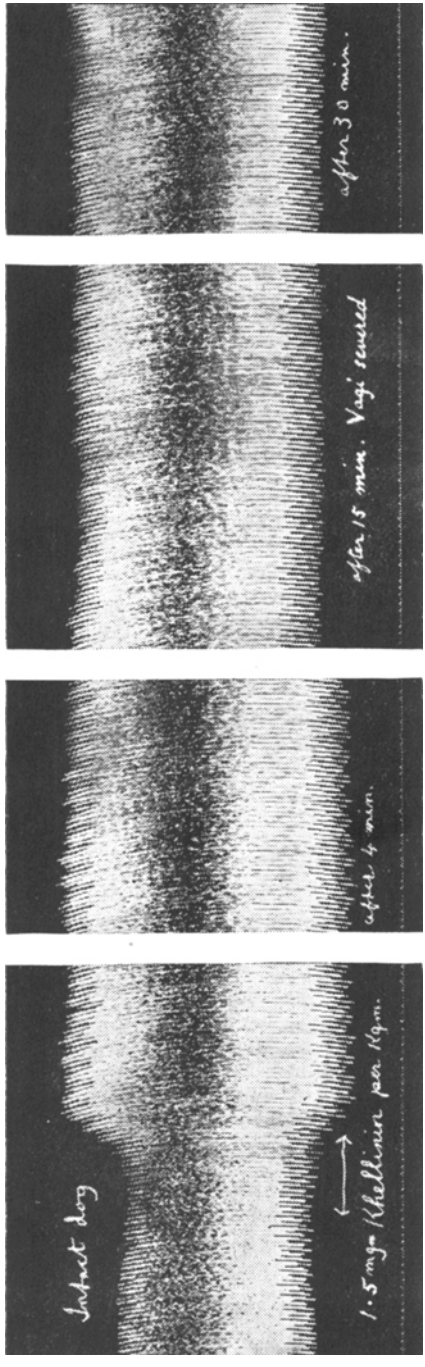

FIG. 2 (reduced).—Intact dog under barbitone sodium and uniform artificial respiration. The ventricular heart beats are recorded with the vagi severed. Intravenous injection of 1·5 mg. of khellinin per kg. increased the contractility of the ventricular muscle producing a more complete systole and a more complete diastole. The rate of beat hardly changed. Record after 4, 15 and 30 minutes of injection is demonstrated.

direct vasoconstricting action on blood vessels. The stimulant action of khellinin on the heart is well exhibited in such small doses and is persistent and rather "selective"—the glycoside, as already described by one of us, has no appreciable action on other organs in even larger doses.

(B) *Toad's heart perfusion:* Several experiments were carried out with various concentrations of visammin (1 : 10,000 to 1 : 100,000) and of khellinin (1 : 20,000 to 1:200,000). All the results were depression with visammin and stimulation with khellinin, with no cumulation. We record (Fig. 4) a triple perfusion, first with Ringer's solution, then with visammin (1 : 35,000 in Ringer's solution) and lastly with the same solution of visammin in which khellinin is dissolved in the same concentration. Depression under visammin is noticeable and the stimulant effect of khellinin is illustrated. In fact this antagonism was referred to by one of us as early as 1932 when it was recorded. "The cardiac depression caused by solutions of visammin is much more marked than equivalent concentrations of tinc-

ture of Ammi Visnaga —the presence of khellinin in the tincture partly accounts for this."

Moreover, Figure 4 demonstrates a greater amplitude of heart beat with visammin and khellinin together than under normal Ringer's solu-

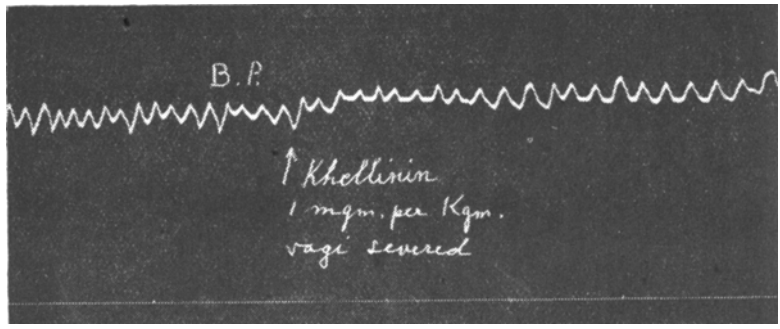

FIG. 3 (reduced).—Blood pressure of a dog under barbitone sodium. Vagi severed. Intravenous injection of 1 mg. of khellinin per kg. raised the blood-pressure.

tion. Presumably, however, one may infer that in the same concentration khellin is more a cardiac stimulant than visammin is a cardiac depressant. The perfusion of a strong solution (1 : 10,000) of visammin (Fig. 5) greatly diminished the degree of systole with arrest of the heart.

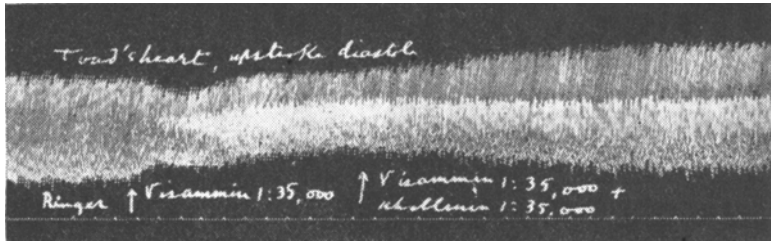

FIG. 4 (reduced).—Triple perfusion of a toad's heart, first with Ringer's solution then with visammin 1:35,000 and lastly with the same solution of visammin in which khellinin is dissolved in the same concentration. Depression of heart beat principally due to a less complete systole with visammin and recovery with stimulation under visammin and khellinin together are demonstrated. The amplitude of beat is greater under visammin and khellinin together than under Ringer's solution. Upstroke diastole.

The continued perfusion of the same solution of visammin to which khellinin was added to a concentration of 1 : 20,000 produced a fairly good recovery of the arrested heart with promotion of systole.

Similar triple heart perfusion experiments were carried out using chloral hydrate, quinine hydrochloride or alcohol in suitable concentrations against the same concentration of each one of these drugs in which khellinin was dissolved (1 : 25,000 to 1 : 50,000), depression of the heart beat was first established with subsequent stimulation in the presence of the glycoside.

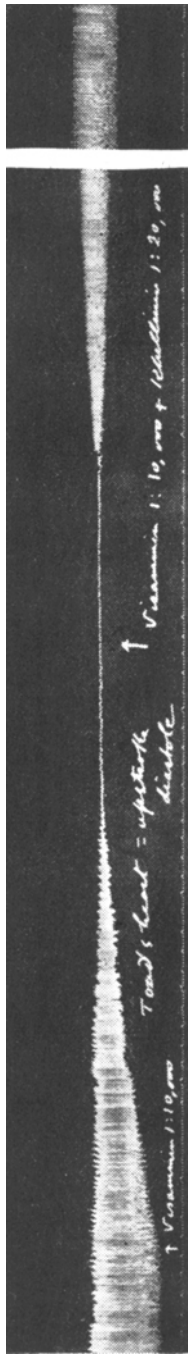

FIG. 5 (reduced).—Triple perfusion of a toad's heart, first with Ringer's solution then with visammin 1:10,000 and lastly with the same solution of visammin in which khellinin 1:20,000 is dissolved. Under visammin alone, systole is greatly reduced with final arrest of the heart, while under visammin and khellinin together, recovery with promotion of systole is seen. Upstroke diastole.

(C) *Isolated rabbit's heart perfusion:* The heart was perfused by a modification of Gunn's method. Pressure of perfusion was kept at 100 to 120 mm. Hg. Outflow was determined every 2 minutes and 5 readings were registered for each change of liquid. In all perfusions partial experimental spasm was induced by the use of barium chloride and for this a concentration of 1:40,000 was maintained throughout—barium chloride Locke's solution was first prefused followed by the same solution of barium chloride containing either visammin (1:40,000) or khellinin (1:40,000) and lastly barium chloride Locke's solution. Only 6 experiments were carried out with the one concentration of visammin and similarly 6 experiments with khellinin giving respectively an average increase in coronary flow of about 200 and 300 per cent. Owing to the limited number of experiments carried out and, moreover, on one concentration only, no conclusion could be drawn as to their relative values in this respect.

Experiments on the intact animal and on the isolated toad's heart similar to those mentioned under (A) and (B) respectively but using various crude samples—obtained from the plant in various stages of impurity—of visammin and of khellinin gave variation and ambiguity of results. In many instances the cardiac depression of visammin or the cardiac stimulation of the glycoside was marked to a great extent. This, however, is very natural. Ammi Visnaga contains quite a number of active bodies. Products which differed from the pure principles by a few degrees in m.pt. gave quite appreciable differences in the response of the heart which is a fairly sensitive organ to these bodies.

Indeed, in addition to determination of m.pt., it would be wise to confirm the purity of material biologically by heart perfusion experiments.

SUMMARY

1. Pure khellinin, m.pt. 175° to 176°C., possesses a persistent, rather selective stimulant action on the heart producing a more

complete systole and a more complete diastole with a corresponding increase in cardiac output. It raises blood pressure. The glycoside is active and doses of 1 to 1·5 mg./kg. of dog by intravenous injection. It increases the coronary flow, and is non-cumulative.

2. Pure visammin, m.pt. 153° to 154°C., depresses the heart, producing principally a less complete systole with diminished cardiac output. The drug is active in doses of 2 to 4 mg./kg. of dog by intravenous injection. It increases the coronary flow.

3. The impurities present in crude samples of khellinin or of visammin influence to an appreciable degree the normal response of the heart to the pure products.

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