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THE EFFECT OF HEPARIN AND ITS COMPONENTS ON FROG HEART

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Abstract—Heparin inhibits the contractions of frog heart. The effect is abolished by calcium but not by atropine or adrenaline. Heparin is able to annul the systolic contracture caused by high calcium concentration. Of the heparin components, the glucuronic acid is responsible for its effect. Glucosamine is ineffective alone, but potentiates the glucosamine effect. Sulfate groupings have most probably a similar effect. The heparin antagonist protamine sulfate also inhibits the beating. The mechanism of the observed effects is discussed.

THE spasmolytic effect of heparin on smooth muscles is known for a long time. This action explains the vasodilatative effect of heparin. Engelberg and Massel[1], Matis and Scheele,[2] Abrahams and Howart[3] and others (see Heilbrunn[4]) have reported this vasodilatative effect and attributed it to a primary action of heparin on the muscles and not to its anticoagulative properties, as no changes in the blood viscosity could be observed in their experiments. The effect of heparin on heart muscle is also discussed in several contributions. Heilbrunn[4], Chaet[5], Kraus *et al.*[6] and Cheymol *et al.*[7] described decreased heart beat under heparin effect. In the present communication authors studied the effect of heparin on frog heart muscle and attempted to elucidate the role of individual components of heparin in the observed effect.

MATERIALS AND METHODS

The effect of the respective substances was tested on isolated frog (*Rana esculenta*) hearts in the winter season. Altogether 180 animals were used in the experiments. The hearts were isolated according to Straub in the usual way. The Straub canule contained 1 ml frog–Ringer solution without or with the substances to be tested. The test solutions were changed repeatedly. The heart beating was recorded on a kimograph. No differences were observed between the reactions of hearts from male or female animals.

RESULTS AND DISCUSSION

Table 1 summarizes the results of the experiments. Under the effect of 3 mg/ml heparin a decrease of contractions is seen as early as after 3 sec and the maximal depressive effect is attained after 30 sec. A slow recovery is elicited by washing the preparation with Ringer solution and a rapid abolishment of the effect is seen under the effect of 1 mg/ml calcium chloride (Fig. 1). The present findings, accordingly, agree with the results of earlier workers describing the relaxation of heart muscle under heparin effect, i.e. its diastolising action.

TABLE 1 EFFECT OF HEPARIN, HEPARIN COMPONENTS AND OTHER SUBSTANCES ON THE BEATING OF FROG HEART.

	Beating decreasing	unchanged	increasing	systolic stop	diastolic stop	Effect of Ringer's	Effect of excessive Ca	Notes
Heparin (Richter) (1 mg = 100 I.U.) 3 mg/ml	+				+	+	+	
Heparin 3 mg/ml + atropin 0·5γ – 10γ/ml	+				+	+		
Heparin 3 mg/ml + adrenalin 1·0γ – 10γ/ml	+				+	+		
Heparin 3 mg/ml + $CaCl_2$ 1·0 mg/ml		+						
Heparin 4 mg/ml + $CaCl_2$ 1·0 mg/ml	+				+	+		
Heparin 3 mg/ml + $CaCl_2$ 0·4 mg/ml	+				+			
+ 0·8 mg/ml $CaCl_2$		+						Amplitude of contractions increasing. Returns to normal.
+3·0 mg/ml $CaCl_2$	+			+		–		Heart stops after a few twitches.
+10 mg/ml heparin			+					Beating resumed

TABLE 1—*continued*

	Beating							
	decreasing	unchanged	increasing	systolic stop	diastolic stop	Effect of Ringer's	Effect of excessive Ca	Notes
$CaCl_2$ 0·6 mg/ml	+			+				Heart stops
+ heparin 10 mg/ml			+			−		Heart resumes action with movements of widely varying amplitudes.
Glucuronic acid 1 mg/ml (d-gluc. ac.)	+				+	+	+	Acts immediately after dissolution
Glucur. ac.-lactone (Acid. glucur. pur. Fluka) 5 mg/ml	+				+	+	+	Acts 25½ hr after dissolution
D glucosamine HCl-Light 5 mg/ml		+						
Glucur. ac.-lactone + glucosamine 5 mg/ml, each	+				+	+	+	Stronger and prompter effect than that of glucuronic acid alone. Acts 140 min after dissolution
Protamine sulfate (Roche) 2 mg/ml	+			+		−	±	Stoppage of heart between systole and diastole
Protamine sulfate 2 mg/ml + atropine 0·5γ − 10γ/ml	+			+		−	−	
Protamine sulfate 2 mg/ml + adrenaline 1·0γ − 10γ/ml	+			+		−	−	

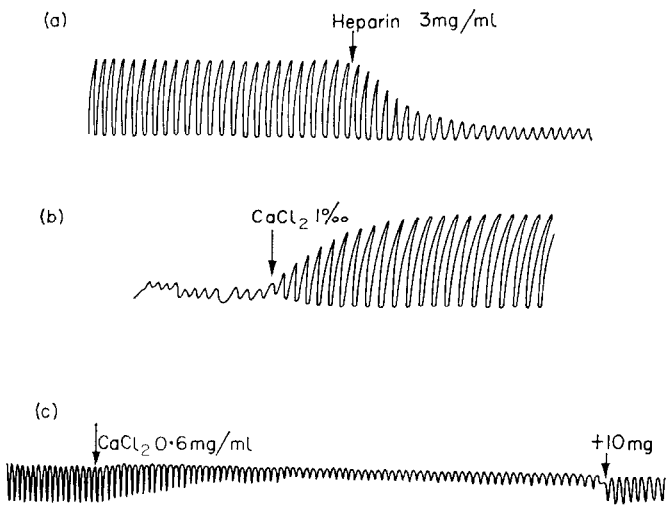

FIG. 1. (a) Effect of heparin 3 mg/ml. (b) Effect of 1 mg/ml $CaCl_2$ on heparin treated heart. (c) Systolic stop elicited by 0·6 mg/ml $CaCl_2$, and its abolition by 10 mg/ml heparin.

The effect of atropine or adrenaline on heparin treated hearts may give some clues as to the mechanism of the effect. These drugs remained ineffective on hearts depressed by heparine thus pointing to a primary action on the muscle itself and against a neurogenic action

The rapid recovery of beating to or even exceeding the initial level after addition of calcium shows that this ion has a genuine antagonistic effect. If the calcium concentration is elevated above 0·1 per cent in the heparin containing Ringer–solution the effect of this ion becomes prevailing. 3·0 mg/ml concentration causes a systolic contracture after a few beats. The contractions start again and become normal after the addition of 10 mg/ml heparin.

The experiment may be performed in the other way as well. A complete cessation of activity can be elicited by addition of 0·6 mg/ml calcium chloride. Large contractions soon appear and a subsequent normalization sets in when the fluid is exchanged with a 10 mg/ml heparin solution (Fig. 1). The stopping with calcium and re-starting with heparin can be repeated several times on the same preparation.

All these results show a clear-cut antagonism of heparin and calcium ions. It seemed, however, necessary to clarify which component of heparin was participating in the action and whether the complete heparin molecule was indispensable for the effect.

Caporro *et al.*[8] showed that heparin caused a heart relaxation even when desulfurated. Chaet[5] ascribed the action to a dialisable component, perhaps a degradation product and not to the complete large molecule.

In the present work the effect of the two monosaccharide components, glucosamine and glucouronic acid, of the heparin (Whistler and Smart[9], Pigman[10]) was tested (Fig. 2). Glucosamine proved to be completely ineffective. Glucuronic acid, however, completely repeated the heparin effect on the heart. One of the tested samples (acid. glucuronicum pur. Fluka) represented the lactone of the compound as proved on the basis of its optical activity and melting point. This substance was ineffective when

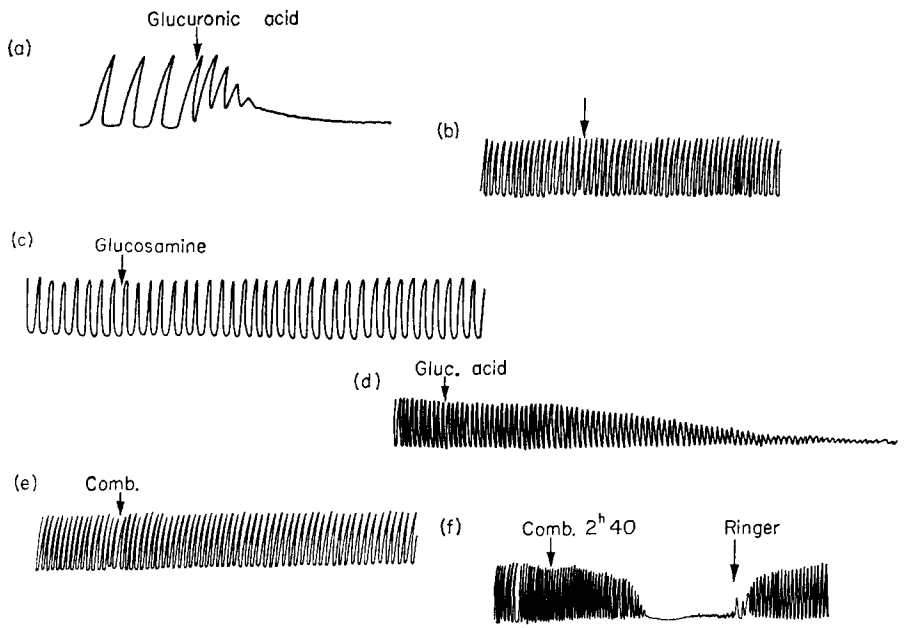

FIG. 2. (a) Effect of freshly dissolved 1 mg/ml glucuronic acid. (b) Effect of 5 mg/ml glucuronic acid lactone after standing for 8 hr. (c) Effect of 5 mg/ml glucosamine after standing for 8 hr. (d) Effect of glucuronic acid lactone 25½ hr after dissolution. (e) Effect of combined solution of glucuronic acid and glucosamine 130 min after dissolution. (f) Effect of combined solution of glucuronic acid and glucosamine 160 min after dissolution.

used freshly dissolved. Its solutions became active after 24 hr standing at room temperature. No explanation of this observation can be given yet. Some authors report on a rotation and increased optical activity of glucuronic-acid when kept in solution for some hours (Bruckner[11]). This phenomenon was used first to explain the development of activity in solutions kept at room temperature for a certain time but polarisation optic analysis revealed no rotation. In spite of numerous confirmations of the fact no explanation can be given for it yet. A disruption of the lactone ring in the dissolved molecule after a certain time might be responsible for the delayed appearance of the activity.

This slowly acting lactone, however, contributed to the elucidation of the role of glucosamine. The lactone of the glucuronic acid and glucosamine together proved to be as ineffective as the lactone alone. The heparin-like relaxing effect appeared, however, after 2 hr 20 min standing in solution in contrast to the 24 hr necessary for the lactone alone to become active.

These experiments point to the glucuronic acid as being the active component of the heparin molecule. Glucosamine which is alone inactive, fastens the transformation of the lactone of glucuronic acid when dissolved together, and increases and fastens the effect of glucuronic acid. The effect of glucuronic acid as well as that of mixed glucuronic acid and glucosamine is antagonized by calcium. It is interesting to note that the calcium induced systolic contracture could be abolished neither with glucuronic acid nor with the mixture of glucuronic acid and glucosamine. Thus a potentiation by sulfate groupings may be assumed as well. There appears a seeming contradiction

to the results of Caporro *et al.*[8] who found that complete and desulphurated heparin had similar activity. The variance in our results may possibly be explained by the fact that Caporro *et al.* did not use hearts in calcium contracture.

To test the effect of the heparin binding protamine sulfate seemed also to be warranted. This substance had a rapid inhibitory effect (Fig. 3). The first signs of inhibition

FIG. 3. Effect of 2 mg/ml protamine sulfate.

were seen after 10 sec and a full cessation of beating after 90–120 sec. The heart stopped in a transitory state between systole and diastole. Atropine and adrenaline gave no, 1 mg/ml calcium gave a slight recovery. No normalization could, however, be observed.

It is impossible to give an unequivocal explanation of the results at present. Both heparin and protamine sulfate inhibit the heart beating. Heparin causes a diastolic stop, whereas its antagonist, protamine sulfate causes a more systolic one. An antagonistic effect of calcium ions is observed primarily on heparin treated hearts and and only slight effect is seen in protamine sulfate treated hearts. In our opinion this points to the role of a certain sol-gel antagonism in these effects. This assumption is supported by the ineffectiveness of neurotropic drugs on both the heparin and protamine sulfate treated hearts, further by the mutual abolishment of calcium or heparin effects. These effects could hardly be explained by a different mechanism. The sol-gel mechanism was already discussed in connection with our earlier experiments (Csaba *et al.*[12]) Further experiments are, however, necessary to prove or disprove definitely our assumption and to clarify the mechanism in detail. Caporro *et al.*[8] attribute the diastolising action of heparin to a removal of calcium from the cell membrane. The experiments of Thomason and Schafield[13] performed with labeled substances showed, however, that heparin causes no removal of calcium. Clark *et al.*[14] assume that calcium is weakly bound to the surface of the heart muscle fibres and the relevant processes take place at the surface of and not within the cell. Only further studies may disclose the mechanism of the effects observed.

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