Table 4. Antagonism of isoproterenol (1 μg/ml)-induced increase in the contractile force of isolated cat heart papillary muscles by compound A or MK-761 (β-adrenoceptor blocking activity)

Treatment	Concentration (μg/ml)	No. of preparations	Contractile force Before treatment (mg ± SE)	Increase after isoproterenol (mg ± SE)
Saline	0.1 ml	14	538 ± 76	+ 994 ± 113
Compound A	0.03	6	427 ± 72	+ 1040 ± 169
Compound A	0.125	6	487 ± 77	+ 647 ± 168
Compound A	0.5	6	473 ± 88	+ 213 ± 63
MK-761	0.03	8	1125 ± 257	+ 790 ± 132
MK-761	0.1	12	747 ± 135	+ 667 ± 93
MK-761	0.3	8	818 ± 130	+ 245 ± 32
MK-761	0.7	8	728 ± 128	+ 140 ± 19
MK-761	1.0	8	600 ± 92	+ 75 ± 19

pine, 0.5 mg/kg i.p., 16–20 h prior to experiments, compound A had positive inotropic activity similar to that observed in normal cats (tables 2 and 3) whereas the positive inotropic effect of MK-761 was nearly abolished by pretreatment with reserpine. This suggests that the positive inotropic effects of MK-761 on papillary muscles from normal cats do not involve direct stimulation of β-adrenoceptors by MK-761 but are mediated by release of catecholamines from the heart muscle.

The β-adrenoceptor blocking activity of the test compounds was estimated by their ability to antagonize the positive inotropic effect of isoproterenol, 1 μg/ml, also on papillary muscles. Test compounds were added at various concentrations to the bath 30 min prior to isoproterenol. Compound A as well as MK-761 reduced the positive inotropic effect of isoproterenol in dose-dependent manner (table 4). There was no significant difference in the potency of the 2 drugs as β-adrenoceptor blocking agents.

The above-described results suggest that both test compounds are effective β-adrenoceptor blocking drugs with acute antihypertensive and cardiac stimulant effects. The cardiac stimulant and acute antihypertensive effects of compound A are likely to be due, at least in part, to direct β-adrenoceptor stimulant activity (ISA) whereas the cardiac stimulant and the acute antihypertensive effects of MK-761 are more likely to be due to a mechanism or mechanisms other than direct stimulation of β-adrenoceptors. This is suggested by the inability of timolol to reduce the antihypertensive effects of MK-761 in SH rats and by reduction of the cardiac stimulant activity of MK-761 by reserpine.

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Failure to identify 'thrombocytolysin' (a spasmogenic factor released from platelets by immunoreaction) with anaphylatoxin

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Summary. Horse platelets release an unidentified smooth muscle contracting substance after lysis by antiserum and complement. Since the active factor (thrombocytolysin) does not produce tachyphylactic response of the guinea-pig ileum it seems that it is not related to anaphylatoxins.

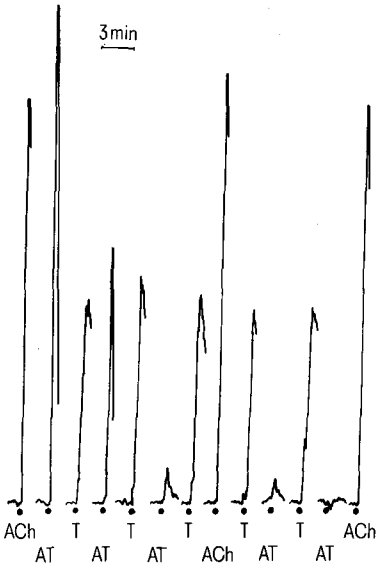
Anti-platelet serum (APS), in the presence of fresh guinea-pig or rabbit serum as a source of complement, releases from washed horse platelets a factor that contracts guinea-pig ileum segments in vitro and causes hypotension in atropinized rabbits[1,2]. A few min after mixing the complete system at 38 °C, there is agglutination of the platelets which settle at the bottom of the tube. As lysis of the platelets progresses a clear supernatant is formed above a transparent viscous mass. Time-course experiments showed that a smooth muscle activity can be detected in aliquots taken from the supernatant. The activity increases, reaches a plateau and eventually drops after 2 h incubation. After contrifugation, the clear, supernatant can be boiled a few min with 2 volumes of ethanol without affecting the activity which is still present in the aqueous phase after removal of the coagulated proteins. An active crude powder was prepared by evaporation of the protein-free filtrate in vacuo at 50 °C (or freeze-drying) which remains active at −20 °C for at least 3 years.

The active principle(s) is water soluble and heat stable in neutral or acidic solutions but is rapidly destroyed by boiling at pH 9 or higher. It is insoluble in organic solvents.

The nature of this smooth muscle active factor has not yet been determined. Pharmacological studies showed that 'thrombocytolysin' (T) is different from histamine, acetylcholine, adenylic acid, ADP, tyramine, bradykinin, 5-HT

(Reid's 'thrombocytin'), Zucker's smooth muscle contractile substance, $K+$ [1-3], taurine, homotaurine, cysteine sulfinic acid, Zn^{2+} ions or prostaglandins[4].

Having in mind that anaphylatoxins (AT) might be formed when APS is incubated with platelets and fresh guinea-pig serum as a source of complement, some experiments were carried out on guinea-pig ileum preparations stimulated by T and rat AT. Platelets were separated from citrated horse blood and used both to raise antibodies in rabbits and produce T. The anaphylatoxin was obtained from rat serum made IM in relation to ε amino-caproic acid and activated by inulin at 37 °C for 1 h[5]. Although inulin, like zymosan, endotoxin, agar, carrageenin, yeast cells, activate complement by the alternative pathway by-passing Cl, the AT released is indistinguishable from that produced by IgG immunocomplex via the classical pathway (C142 mechanism).



Guinea-pig ileum isolated preparation bathed (10 ml) in Krebs-bicarbonate solution gassed with 5% CO_2 in O_2 at 37 °C. ACh = 2.5×10^{-8} M acetylcholine; AT = anaphylatoxin solution (5 µl/ml); T = 'thrombocytolysin' from horse platelets (2 µl/ml).

When successive doses of AT were applied at short intervals to a guinea-pig ileum preparation bathed in Krebs-bicarbonate physiological solution, tachyphylaxis rapidly developed, the tissue being partially or completely desensitized after a few such applications. A typical experiment is summarized in the figure. Although desensitized after 5 successive additions of AT the preparation still responded regularly not only to T but to calibrated doses of acetylcholine as well. As one of the most important features of the anaphylatoxic activities on smooth muscle preparations is a cross-desensitization, it seems that an AT can be ruled out as the major active component in partially purified T preparations.

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Cytochalasin D is able to mimic the effects of phalloidin on the rat liver

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Summary. Cytochalasin D induces a strong potassium release in the isolated perfused rat liver and leads to vacuolization of the liver parenchymal cells. These effects are similar to the action of phalloidin on the rat liver. Since phalloidin and cytochalasin act in a different way on microfilaments, it is suggested that any disturbance of the function of microfilaments can induce these effects.

Phalloidin, a toxin, acting specifically on actin filaments in liver parenchymal cells[10], induces vacuole formation and potassium release in the isolated perfused rat liver[3]. It is not clear, however, in which way the molecular effect of phalloidin causes the pharmacological phenomena. Therefore we studied the effect of cytochalasin D, another drug interfering with the function of microfilaments.

Material and methods. The liver of male Wistar rats (mean animal weight 200 g) was perfused at 27 °C with a recirculating medium using a membrane oxygenator. The perfusion medium (vol. 50 ml) contained 8% dextran but no red cells. The perfusion rate was 3-4 ml/g/min. Details of the method have been described[4]. For light and electron microscopic investigation, livers were fixed by perfusion with 2.5% glutardialdehyde in the buffer solution used for preparing the perfusion medium, postfixed with 1% OsO_4 and embedded in ERL (Serva, Heidelberg). Cytochalasins (Aldrich, Milwaukee) were dissolved in dimethylsulfoxide (20 mg/ml). For i.v. application, additional DMSO (about 100 µl/kg) and Cremophor EL® (about 100 µl/kg; BASF, Ludwigshafen) were added. The solvents alone had no effect on the parameter investigated.

Results and discussion. When cytochalasin D (1 mg) was applied to the isolated perfused rat liver a potassium release of about 45 µEq/g liver could be observed within 60 min (figure 1, a). Between 60 and 120 min, a small amount of the potassium previously released was taken up again by the liver. This potassium uptake was not observed after a dose of 2 mg cytochalasin D (2 experiments, not shown). In contrast, cytochalasin B[4], even after a dose of 4 mg, induced only a small potassium release (up to 15 µEq/g) which was completely reversible within 60-70 min (2 experiments, not shown). The bile flow ceased after application of cytochalasin D within 6-10 min. The potassium release from the cytochalasin D treated liver was prevented by a high Mg^{2+} concentration (40 mM) in the perfusion medium (figure 1, a). Under the same conditions, the potassium release after phalloidin poisoning was also inhibited[5].

Application of hexobendine or 4,7-phenanthroline (15-25 mg) to the perfusion medium, 60 or 120 min after cytochalasin D led to a nearly complete re-uptake of the potassium previously released (figure 1, b). These drugs were similarly effective in the phalloidin-poisoned liver[6].