# Hemodynamic Effects of Group A *Streptococcus* Mucopeptide (37733)

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(Introduced by P. P. Foà)

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Streptococcal mucopeptide plays not only an important role in securing the rigidity of the cell wall (4), but has also potent biologic properties, some of which resemble those of endotoxin. In mice, streptococcal mucopeptide induces a nonspecific resistance to experimental streptococcal infections and, when injected intravenously, it provokes a pyrogenic response. In addition, streptococcal mucopeptide has a potent effect on blood platelets, causes a local Shwartzman reaction and cardiac lesions (5, 6, 10, 11).

We have investigated the hemodynamic changes that follow the intravenous administration of streptococcal mucopeptide in cats and in rabbits.

*Methods.* Rabbits were anesthetized with urethane (1.2 g/kg) or with pentobarbital (30 mg/kg). Cats were anesthetized with alpha chloralose (100 mg/kg).

Arterial blood pressure was measured in the femoral artery with a mercury manometer or with a Elema–Schönander pressure transducer. The heart rate was obtained from ECG records. The cardiac output was measured by a thermodilution technique described previously (7). The thermal indicator, saline at room temperature, was infused into the right atrium at a constant speed, and matched thermistor catheters were introduced into the pulmonary artery via the right jugular vein. Thermodilution curves were recorded on a four-channel jet-writing oscillograph and the area under the curves was calculated by planimetry. Cats were decapitated between the 1st and 2nd cervical vertebra using an acraseur and the animals were ventilated artificially. To detect a possible release of pharmacologically active substances into the circulation, the autoperifusion technique of Vane (12) was used. In this method, a continuous stream of arterial blood is superfused over an isolated rat stomach, a guinea pig ileum, or a rat ascending colon by means of a digital pump and returned to the animal through a cannula in a jugular vein.

Thrombocytopenia was produced in rabbits by the intravenous administration of dog plasma containing antibodies against rabbit platelets.

Group A, type 6 *Streptococcus* strain S 43/100 was used for the preparation of mucopeptide. Bacterial sediment from a 16-hr broth culture was washed in a nonpyrogenic NaCl solution, and the bacteria were submitted to mechanical disintegration in a Mickle apparatus, using ballotini glass beads. The cell walls were separated from cytoplasmatic membranes and intracellular components by differential centrifugation. Further purification was achieved by treatment with proteolytic enzymes, RNase and DNase.

The separation of carbohydrate and mucopeptide from the deproteinized cell wall was performed by extraction with hot formamide, according to the procedure of Krause and McCarty (4). The lyophylized mucopeptide was suspended in apyrogenic NaCl solution at a concentration of 1 mg/ml, immediately before the experiments. The suspension was submitted to ultrasonic treatment in an MSE ultrasonic disintegrator (20 kc/s for 30 min). The degree of solubilization of mucopeptide was expressed in terms of the hexosamine content of the supernatant fluid after centrifugation at 6000*g* for 30 min. Under these conditions, the supernatant fluids contained 50% of the total hexosamine present in the starting material. The doses of solubilized mucopeptide were prepared by diluting

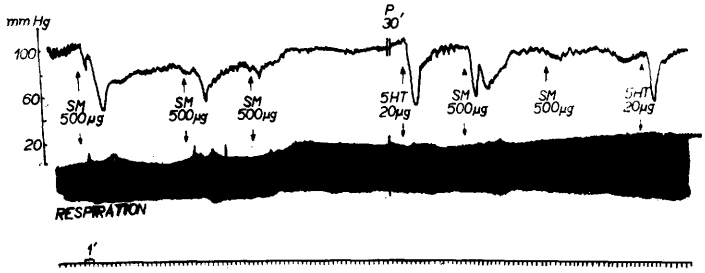


FIG. 1. Effect of streptococcal mucopeptide (SM) and 5-hydroxytryptamine (5-HT) on the blood pressure of a rabbit (2.5 kg) under urethane anesthesia.

the disintegrated material with nonpyrogenic NaCl solution.

*Results.* In seven rabbits anesthetized with urethane, the administration of mucopeptide, in doses of 200 μg/kg, invariably produced marked hypotension followed by slow recovery. Repeated administration of mucopeptide at 10-min intervals gradually led to almost complete loss of its hypotensive potency. This was restored after an interval of not less than 30 min. The same degree of hypotension was achieved with 5-hydroxytryptamine (5-HT) in doses of 8 μg/kg, but in this case the recovery was more rapid (Fig. 1). Seven rabbits anesthetized with pentobarbital appeared to be more sensitive to mucopeptide. In these animals, the blood pressure did not return to normal for about 60 min and the hypotension was accompanied by a considerable decrease in cardiac output (Fig. 2). The changes of heart rate were not statistically significant.

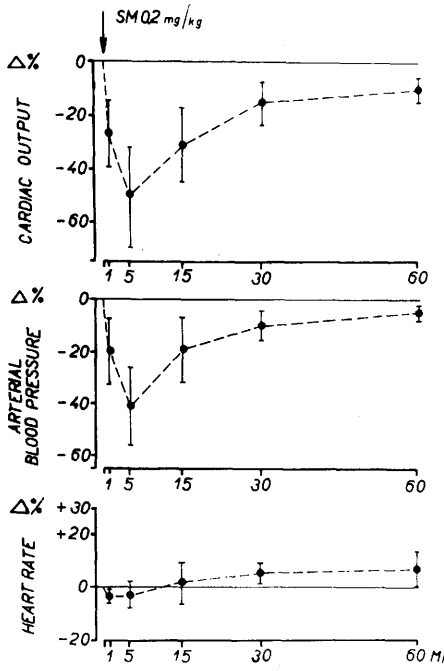


FIG. 2. Effect of streptococcal mucopeptide (SM) on cardiac output, blood pressure, and heart rate of rabbits under pentobarbital anesthesia. Mean values from seven animals with their limits of confidence, $p = 0.95$.

The circulatory changes described above were accompanied by a fall in the thrombocyte count that reached a maximum of 85% during the first 10 min and had not returned to normal 24 hr later (Fig. 3).

To obtain some evidence about the possible role of 5-HT in the observed blood pressure fall, the experiments were repeated in the 7 thrombocytopenic animals. As can be seen from Fig. 4, these animals were sensitive to exogenous 5-HT, but the initial sharp hypotensive effect seen after mucopeptide administration in normal rabbits was completely abolished. The results of a typical autoperifusion experiment designed to investigate the possible release of the hypotensive substances are shown in Fig. 5. It may be seen that rat stomach, the guinea pig ileum and, to some extent, the rat colon contracted as the result of pharmacologically active substances released into the circulation by the mucopeptide.

Experiments in cats demonstrated that this species is very susceptible to the hypotensive action of mucopeptide. In the cat, an almost immediate profound fall of blood pressure was followed by a slower longer lasting

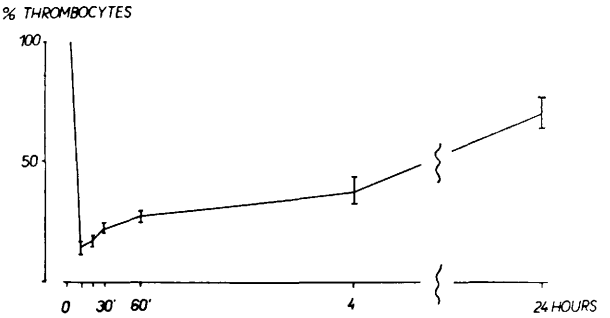


FIG. 3. Thrombocyte count after the administration of 200 μg/kg of streptococcal mucopeptide. Mean values from seven rabbits with their limits of confidence, $p = 0.95$.

phase of hypotension. Bilateral vagotomy reversed the hypotensive response induced by mucopeptide. The response to 5-HT was changed in the same manner. A typical record is shown in Fig. 6.

Decapitation of cats did not change their response to mucopeptide.

*Discussion.* Streptococcal cell wall mucopeptide injected intravenously into rabbits and cats caused an abrupt fall of arterial blood pressure and a decrease of cardiac output without significant changes in heart rate and calculated peripheral resistance. The duration of these effects was dependent upon the dose and the type of anesthesia. Thus, in this respect, these effects resemble those of endotoxins derived from gram-negative bacteria (2, 8, 10).

Percentage decrease of cardiac output generally exceeded the percentage decrease of mean arterial blood pressure; thus both myocardial depression and peripheral vasodilatation, including blood pooling, may have taken place.

A primary question is to what extent the observed hemodynamic changes were the results of a direct effect of the mucopeptide and to what extent they were mediated through the release of other active substances. In this respect, we have obtained some evidence for the participation of hypotensive substances. The marked thrombocytopenia observed after mucopeptide administration and the marked change in the hypotensive response of thrombocytopenic animals suggest that active substances may be released from blood platelets.

This assumption is substantiated further by our previous finding that mucopeptide and platelets interact *in vitro*, releasing 5-HT (9), as well as by the results of the autoperifusion experiments demonstrating the release of substances that stimulate the contraction of guinea pig ileum and rat stomach. Previous studies of our group clearly indicated that the biological effects of mucopeptide preparation described in our paper cannot be due to endotoxin contamination. Rabbits made tolerant to endotoxin fever continued to respond with hyperthermia to mucopeptide injection and the fever induced by mucopeptide was inhibited by specific mucopeptide

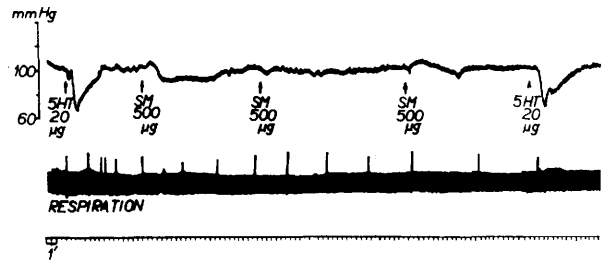


FIG. 4. Effect of 5-hydroxytryptamine (5-HT) and streptococcal mucopeptide (SM) on the blood pressure of a thrombocytopenic rabbit (2.5 kg) under urethane anesthesia.

antibodies (10). Moreover, endotoxin and mucopeptide have different effects on sleep cycle of rat, where mucopeptide is more effective than endotoxin, while the latter is more pyrogenic (3). After vagotomy, in cats, mucopeptide injections resulted in a rise rather than a decline in blood pressure. Vagotomy causes a similar reversal of the response to 5-HT in cats, but not in dogs. This species difference is not surprising since it is known that 5-HT stimulates all vagal fibers only in the cat (1). Decapitation did not prevent the hypotensive response to streptococcal mucopeptide in cats. Thus, the central nervous system does not appear to play an important role in the primary effect of mucopeptide. Probably the marked pharmacodynamic activity of streptococcal cell wall mucopeptide is the result of direct effects on sensitive

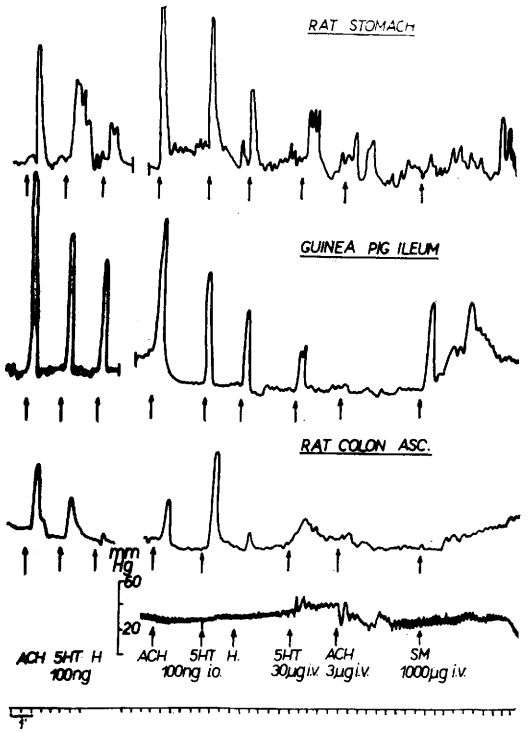


FIG. 5. Perifusion of isolated organs with the blood of a rabbit (2.5 kg) under urethane anesthesia. I.O. indicates the effects of acetylcholine (ACh), 5-hydroxytryptamine (5-HT), histamine (H), and streptococcal mucopeptide (SM) on isolated rat stomach, rat colon, and guinea pig ileum. I.V. indicates the results of intravenous injections.

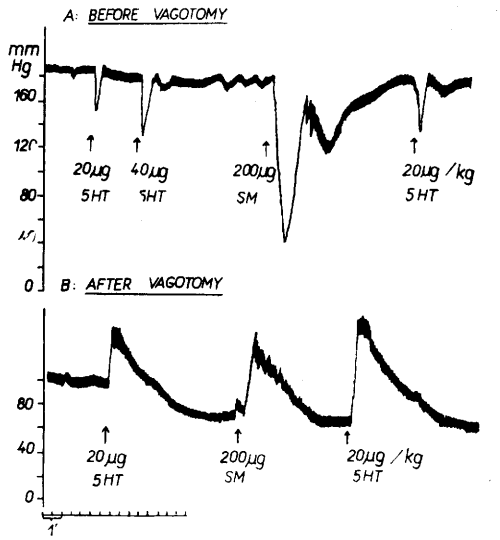


FIG. 6. Effect of vagotomy on the blood pressure response of a cat (1 kg) injected with 5-hydroxytryptamine (5-HT) and streptococcal mucopeptide (SM).

structures and combined with the release of pharmacologically active substances.

*Summary.* Streptococcal cell wall mucopeptide administered intravenously to experimental animals provoked marked circulatory changes. A fall of arterial blood pressure and a decrease of cardiac output were observed. These changes are similar to those reported after the administration of bacterial lipopolysaccharide. Some evidence suggests that both a direct effect of mucopeptide on sensitive structures and a release of pharmacologically active substances may play a role in the hemodynamic disturbances. On the other hand, the central nervous system probably plays no important role in the circulatory effect of the mucopeptide.

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