PHYSIOLOGY

The Direction of Vasomotor Effects of Thrombin is Regulated by Vascular Tone

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Experiments on circular preparations of rat aortic arch show that after an increase in vascular tone thrombin-induced vasoconstriction is replaced by vasodilation. This effect is observed when vascular tone is modified with norepinephrine or prostaglandin $F_{2\alpha}$. Mechanical de-endothelialization abolishes dilation and has no effect on constriction. Dilation is blocked by incubation with nitroarginine.

Key Words: *thrombin; endothelium; vascular tone*

Morphological and functional damage to the vascular wall initiates blood coagulation aimed at thrombus formation and vasoconstriction. Vasoconstriction is determined by a number of vasoactive substances formed in the process of blood coagulation [8]. Thrombin, a compound with a wide spectrum of biological activity, is of particular interest since it is involved in all stages of homeostasis by interacting with plasma proteins, blood cells, and vascular wall [2]. Thrombin induces both constriction and dilation of blood vessels, the response being species- and organospecific [6,11]. It was found that both effects of thrombin are determined by the active center and are realized via specific receptors [2,4,7]. Vasoconstriction is an endothelium-independent and Ca^{2+}-dependent response involving protein kinase C [4,7, 10]. Vasodilation is an endothelium-dependent reaction realized with participation of nitric oxide [9].

Out objective was to find out how modifications of vascular tone influence the vasomotor effects of thrombin.

MATERIALS AND METHODS

The standard methods of measuring tonic activity in isolated circular preparation of rat aortic arch in Krebs—Henseleit buffer were used [1]. Measurements were performed with a Harward Apparatus Isometric Muscle Transducer (Model 363) and a Hitachi-056 automatic recorder. Stable tone of the preparation was attained with a 1000 mg load, after which maximum vasoconstriction was induced with 1 μM norepinephrine (NE). Papaverine (10 μM, maximum dilation) was added at the end of each experiment. The desired vascular tone was achieved by adding varied concentrations of NE and prostaglandin $F_{2\alpha}$ (PG) to the incubation medium. Vascular tone was expressed as a percentage, assuming the range between the maximum constriction and dilation as 100%. Constriction and dilation of the preparation induced by thrombin and acetylcholine (AC) were also expressed as a percentage, assuming the maximum possible constrictor and dilatory effects at the given vascular tone as 100%. Papaverine, NE, Nω-nitro-L-arginine (nARG) were from Sigma, and thrombin (Test-Thrombin) was from Boehringer.

RESULTS

The addition of thrombin in "physiological" concentration (0.1, U/ml) induced constriction of circular aortic preparation with residual tone (after a 1000-mg load, Fig. 1). An increase in vascular tone with NE to 30% of the maximum level slightly re-

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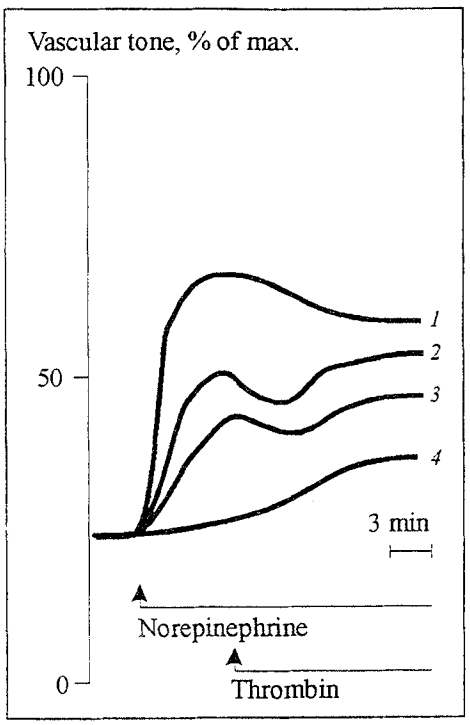

Fig. 1. Relationship between magnitude and direction of thrombin vasomotor effects and vascular tone. Norepinephrine concentration: 1 (3), 10 (2), and 100 nM (1); 4) spontaneous tone.

duced the two-phase vasomotor reaction, while a 50% increase induced a two-phase vasomotor reaction: the early transitory dilation phase was replaced by a longer constriction phase (Fig. 1). At high vascular tone (70% of the maximum), thrombin induced only dilatory response. In order to check up the hypothesis that vascular tone but not specific interactions between thrombin and NE changes the direction of thrombin effects, a separate series of experiments with PG was performed. Modification of vascular tone with PG induced the same transformations of the vasomotor response to thrombin as were observed with NE. Interestingly, the response to thrombin was similar at similar vascular tones created with PG and NE. Proceeding from the nature of constrictory and dilatory responses to thrombin [7,9], it can be suggested that direct action of thrombin on the smooth muscle predominates at a low vascular tone, while at a high vascular tone the effect of thrombin is mediated by the endothelium. In order to check up this suggestion the two-phase reaction to thrombin observed at 50% tone of the preparation was studied in experiments with mechanical deendothelialization and blockade of the nitric oxide synthesis with nARG [3]. De-endothelialization, the efficiency of which was controlled by blockade of AC-induced dilation [5], abolished the dilatory phase in response to thrombin. The two-phase response became a constrictor reaction (Fig. 2). Nitroarginine produced a similar, although less pronounced, effect.

Thus, the direction of vasomotor reactions of rat aortic preparations to low concentrations of thrombin is determined by the tone of vascular smooth muscles and integrity of endothelial lining. As the tone of blood vessels with intact endothelium increases, the direct constrictory effect of thrombin on the smooth

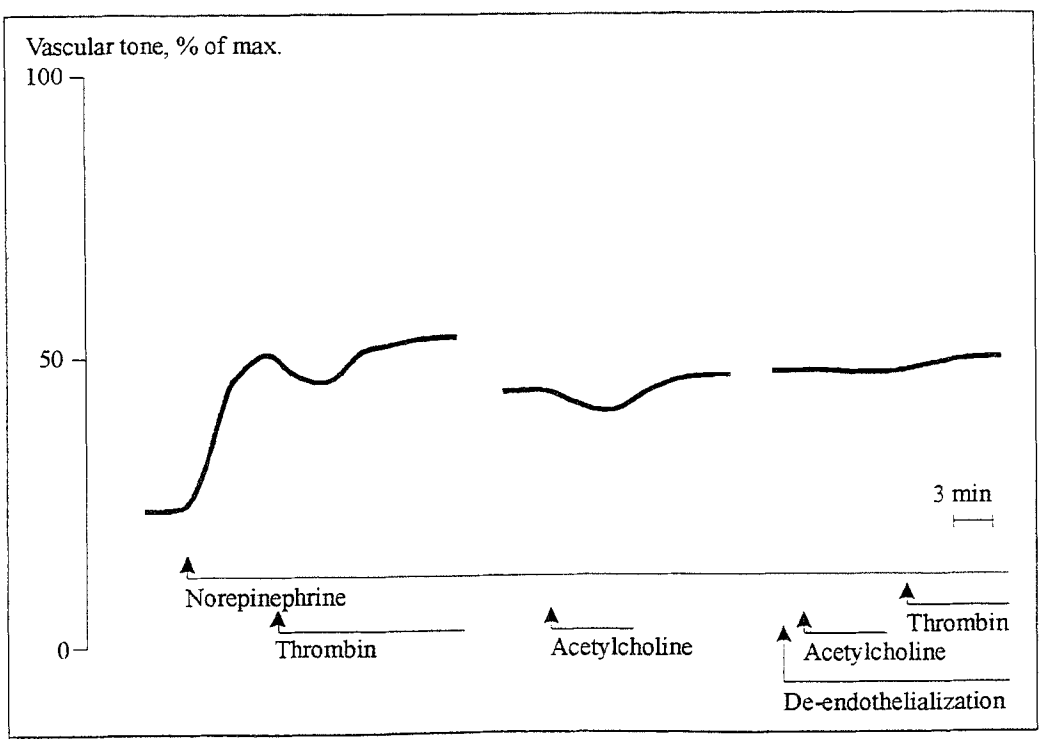

Fig. 2. Effect of de-endothelialization on the magnitude and direction of the vasomotor effect of thrombin. Norepinephrine 10 nM; acetylcholine 5 nM.

muscle is replaced by endothelium-mediated dilatory effect. Presumably, this is due to endothelial "packing" by increased vascular tone, which reduces the accessibility of smooth muscle to thrombin. This mechanism does not operate after endothelial injury, when vasoconstriction predominates. Our results suggest that thrombin stabilizes vascular tone by decreasing it in vascular spasm and increasing it upon the tone loss.

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