# PHYSIOLOGY

# Vascular Reactivity in NISAG Hypertensive Rats

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*In vitro* experiments on isolated segments of abdominal aorta of NISAG hypertensive rats with assessment of $^{32}P$ incorporation showed that hypertension in these rats is developed as due to enhanced metabolism of phosphatidylinositol phosphates under the effect of norepinephrine resulting in higher vascular reactivity to norepinephrine.

**Key Words:** *arterial hypertension; stress; vascular reactivity*

Increased reactivity of the muscle type arteries to sympathetic influences may be a mechanism underlying arterial hypertension. NISAG rats are a new experimental model of arterial hypertension that simulates the stress-dependent form of human arterial hypertension [1,4]. There is evidence that variation in the stress reactivity in these rats is related to disturbances of central noradrenergic mechanisms that participate in the regulation of arterial pressure (AP) and in stress reactions [5]. However, this does not exclude the possibility that modified vascular reactivity also contributes to the maintenance of increased AP. Our aim was to compare reactivity of vascular system in normotensive and NISAG rats.

## MATERIALS AND METHODS

The study was carried out on 10 male NISAG and 10 male Wistar rats (320 g) at the age of 5-6 months. Reaction of AP to intra-arterial administration of norepinephrine (NE, 2 μg/kg) was measured in an acute experiment under Nembutal anesthesia. A polyethylene catheter was threaded into abdominal aorta via the tail artery. Mean AP was recorded with an Mingograph-42 electromanometer for 10 min before and 60 min after NE administration. The reactivity of the muscle type arteries to NE was studied *in vitro* in an isolated segment of the tail artery. An arterial segment was perfused by oxygenated Krebs—Henseleit solution at 37°C under a constant pressure of 70 mm Hg [2]. NE (5 ml) was introduced into the perfusion system as a bolus in concentration of 0.01 μg/kg dissolved in 5 ml of perfusion solution. The effects were recorded for 10 min as variations of the flow in the artery. The synthesis of phosphatidylinositol phosphates (PIP) was studied *in vitro* in an isolated segment of abdominal aorta by the intensity of $^{32}P$ incorporation. The abdominal aorta was incubated for 90 min in the Krebs medium (saturated with 95% $CO_2$ and 5% $O_2$ mixture) which contained $^{32}P$-$P_i$ (1 mCi/ml) and NE ($5\times10^{-7}$ M). Then the medium was removed, and incubation was arrested by addition of methanol. The tissue was homogenized, and phospholipids were extracted [3,6]. Extracted and dried lipids were dissolved in a chloroform:methanol mixture (1:2). An aliquot was withdrawn to determine the phosphorus concentration [6]. Phosphatidylinositides were separated by thin-layer chromatography in the chloroform:methanol:3.3 N $NH_4OH$ system.

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## RESULTS

The amplitude and duration of AP increment in rats of both strains caused by intra-arterial administration of NE were approximately the same (Fig. 1), although the rats had a marked difference in the initial levels of AP (32 mm Hg). These differences in AP impeded evaluation of vascular reactivity by reaction to a vasoconstrictor, which evidently depended on the initial vascular tone. If the reactivity is enhanced, which most probably takes place in hypertensive rats, one should expect a smaller increment in AP in response to NE in comparison with the control Wistar rats.

More definite information on vascular reactivity was obtained by *in vitro* evaluation of the reaction to EN of isolated segment of the tail artery. Addition of NE to the perfusion medium resulted in constriction of the segment and caused a proportional decrease in the flow of perfused solution through it. The difference of the flow decrements in the rats of both strains was essential and significant. The flow decreased by 3.32 and 1.27 times in the arteries of NISAG and Wistar rats, respectively ($t$=2.17, $df$=18, $p$<0.05). Thus, the hypothesis about an enhanced reactivity of the muscle type arteries in hypertensive NISAG rats in comparison with Wistar rats was supported by the *in vitro* experiments on isolated arterial segments.

Different mechanisms may be responsible for the enhancement of vascular reactivity. Sympathetic regulation of vascular tone involves $\alpha_1$-, $\alpha_2$-, and β-adrenoreceptors of the smooth muscle cells. Interaction of $\alpha_1$-adrenoreceptors with the specific ligand leads to vasoconstriction which is caused by activation of second messengers (phosphatidylinositol diphosphate ($PIP_2$)-specific phospholipase C), release of diacylglycerol and inositol trisphosphate, and an increase in the intracellular calcium [7].

The contribution of $\alpha_1$-adrenoreceptors to the increase in vascular reactivity in NISAG rats was assessed by activation of phospholipase C induced by NE. Activation of $PIP_2$-specific phospholipase C caused by interaction of $\alpha_1$-adrenoreceptors with NE should stimulate incorporation of $^{32}P$ into phosphatidylinositol monophosphate (PIP), which is produced by phosphorylation of phosphatidylinositol, and into $PIP_2$, which is the product of PIP phosphorylation [3]. Addition of NE in the specified concentration to isolated abdominal aorta enhanced incorporation of $^{32}P$ into PIP and $PIP_2$ in the hypertensive rats, while it produced virtually no effect in the aorta of normotensive Wistar rats (Fig. 2). Enhancement of $^{32}P$-metabolism of phosphatidylinositides in the aorta of hypertensive rats attests to a

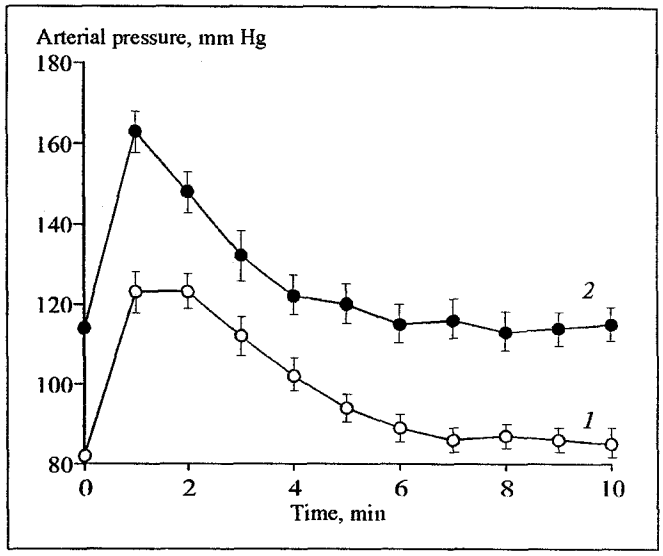


**Fig. 1.** Variations of mean arterial pressure in (*1*) Wistar and (*2*) NISAG rats in response to intra-arterial administration of norepinephrine (2 μg/kg).

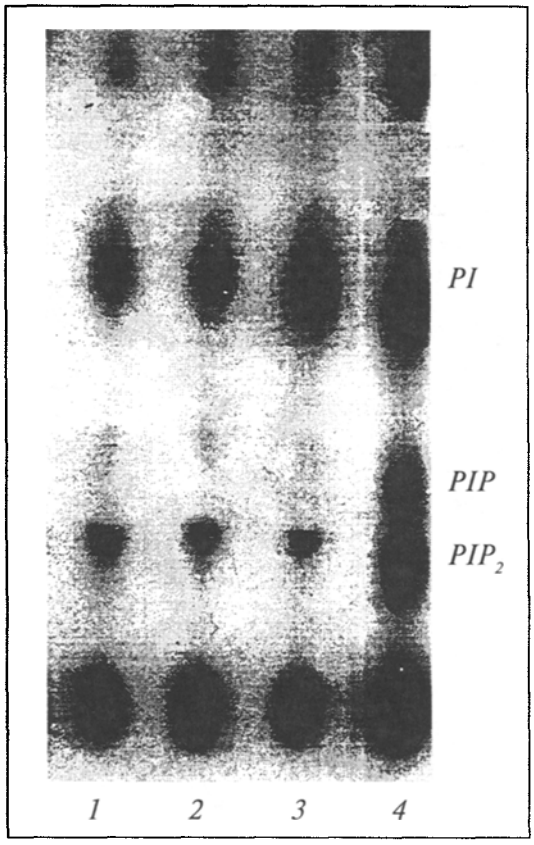


**Fig. 2.** Effect of norepinephrine on $^{32}P$-labeled polyphosphoinositides of aorta in (*1,2*) Wistar and (*3,4*) NISAG rats. The fragments of isolated aorta were incubated (*1,3*) in the absence and (*2,4*) in the presence of norepinephrine ($5\times10^{-7}$ M) in the medium containing $^{32}P$-orthophosphate (1 mCi/ml). The chromatogram shows phosphatidylinositol (PI), phosphatidylinositol monophosphate (PIP), and phosphatidylinositol diphosphate ($PIP_2$).

higher level of phospholipase C-induced activity in the vessels of hypertensive rats. Activation of $PIP_2$ hydrolysis leads to the release of inositol trisphosphate, which increases the intracellular $Ca^{2+}$ and results in vasoconstriction.

Therefore, increased sensitivity of NISAG rats arteries to NA in comparison with normotensive Wistar rats, which is mediated by the system of PIP synthesis, is probably one of the mechanisms that control rising of AP during emotional stress, which is accompanied by an enhanced release of catecholamines.

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