ANTAGONISM BY PHENTOLAMINE AND YOHIMBINE NOT HYDRALAZINE ON NORADRENALINE-INDUCED HYPERTENSION IN RAT

Prasert Songkittiguna and Giatbungon Jindagul

**Department of Pharmacology,
Faculty of Dentistry, Chulalongkorn University,
Bangkok 10330, Thailand**

Noradrenaline (10 μg, IV) increases blood pressure of anaesthetized rats with little effect on heart rate. Pretreatment of the rat with hydralazine (2 mg/kg IV) could not inhibit the noradrenaline-induced hypertension in the rats. Both phentolamine (2 mg/kg IV) and yohimbine (0.25 mg/kg IV) not only reduce the blood pressure of anaesthetized rats significantly ($p < 0.05$) but also inhibits the hypertensive effect of noradrenaline (10 μg, IV). Phentolamine (2 mg/kg IV), neither hydralazine nor yohimbine pretreatment, significantly decreased the heart rate of the anaesthetized rats ($p < 0.05$) from the predrug value. The findings suggest, therefore, that hydralazine is not suitable for the treatment of noradrenaline-induced hypertension.

Gross and coworkers have shown since 1950 that hydralazine is the most effective antihypersive agent of the derivatives. Hydralazine has been used widely to treat hypertension that occurs during pregnancy. Parenteral administration of hydralazine has also been used for the treatment of hypertensive emergencies (Gerber and Nies, 1991). There has been no report on the effects of hydralazine on noradrenaline-induced hypertension in rat. Therefore, the comparative study on the antagonistic effect of phentolamine, yohimbine and hydralazine on the noradrenaline-induced hypertension in anaesthetizied rat is is carried out with a view to shed some more indication of hydralazine on antihypertensive treatment.

MATERIALS AND METHODS

Wistar rats of either sex weighing 250-300 g were anaesthetized with urethane (10 mg/kg). Urethane 25% in normal saline solution was injected intraperitoneally. The trachea was cannulated to maintain the animal on positive pressure ventilation in order to avoid any complicating effects of respiration. The blood pressure was recorded directly from carotid artery on a Grass Polygraph Model 79 (FT03). The heart rate was recorded from tachometer. The freshly-prepared hydralazine (2 mg/kg) in normal saline solution was injected into the femoral vein. Phentolamine hydrochloride, yohimbine also freshly-prepared in normal saline solution. The amount of each drug injection should not exceed 1 ml. Noradrenaline was also preparedbefore use, it was mixed with antioxidant (ascorbic acid) and immersed in ice throughout the experiments. Noradrenaline (10 μg) was injected intravenously; the blood pressure and heart rate were recorded for 15 min. After the action of noradrenaline wore off, the blood pressure and heart rate had reached the

Group	changes of mean blood pressure (%) and heart rate in the parentheses Standard error of mean (s.e.m)				N
	Noradrenaline alone (10 μg)	Noradrenaline 10 μg after			
		Yohimbine (0.25 mg/kg)	Phentolamine (2 mg/kg)	Hydralazine (2 mg/kg)	
1.	64.9 ± 5.0	72.8 ± 6.9	-	-	7
2.	71.5 ± 6.9	(−1.4 ±	30.8 ± 6.4	-	6
3.	64.0 ± 4.9	(2.3 ± 1.1)	105.9 ± 8.6	7 (−7.2 ± 5.9)	

Table 1. The percent changes of mean blood pressure and heart rate produced by noradrenaline alone and by noradrenline after yohimbine or phentolamine or hydralazine. N = number of experliments

equilibrium, hydralazine 2 mg/kg IV was introduced the blood pressure and heart rate were recorded for 30 min, then 10 μg of noradrenaline was injected intravenously. The blood pressure and heart rate was recorded for 30 min. The same procedure was performed using phentolamine (2 mg/kg IV) or yohimbine (0.25 mg/kg IV) in the place of hydralazine. The experiments were performed in separate experiments. Noradrenaline (10 μg IV) was injected before and after phentolamine or yohimbine, then blood pressure and heart rate were recorded for 30 min. statistical analysis of results

One way analysis of variance was performed on a group data followed by the use of paired or unpaired Student's t-test (where appropiate).

Drugs

Hydralazine hydrochloride (Ciba-Geigy); phentolamine hydrochloride (Ciba-Geigy); yohimbine (Sigma); urethane (May & Baker); (+) noradrenaline hydrochloride (Sigma).

RESULTS

In the first group of seven rats, when noradrenaline (10 μg, IV) was given, the mean blood pressure was risen to 64.9% (s.e.m. 5.0) of the predrug value. Prior to the same amount of noradrenaline was given, hohimbic (0.25 mg/kg, IV) had been introduced, the percentage rise in mean blood pressure was 72.8 (s.e.m. 6.9) whereas the heart rate was depressed 1.4% (s.e.m. 0.5). The second group of six rats, noradrenaline (10 μg) alone raised the mean blood pressure to 71.5% (s.e.m. 6.9). After phentolamine (2 mg/kg, IV), noradrenaline (10 μg) raised the mean blood pressure to 30.8% (s.e.m. 6.4) while the heart rate was 2.3% (s.e.m. 1.1). In the third group of seven rats, noradrenaline by itself increased the mean blood pressure to 64.0% (s.e.m. 4.9). After hydralazine (2 mg/kg, IV), noradrenaline (10 μg) increased the mean blood pressure up to 105.9% (s.e.m. 8.6) and the heart rate was depressed 7.2% (s.e.m. 5.9).

DISCUSSION

As mentioned in the introduction, no evidence that hydralazine affects membrane channeis or cGMP as has been reported for other vasodilator (Weiss, et al., 1990). Furthermore, Spokas, et al. reported, in 1983, that the mechanism of direct relaxation of

arteriolar smooth muscle by hydralazine is not clear; numerous hypothesis have been put forth. Part of the vascular relaxation caused by hydralazine is dependent on the presence of endothelium. In addition, nitric oxide can be generated from hydralazine in vitro (Kruszyna, et al., 1987). The direct acting, nitrogenous vasodilators cause red blood cells to generate nitric oxide (Gerber and Nies, 1991). In addition the inhibition of catecholamine biosynthesis by hydralazine has been documented (Liu, et al., 1974; Songkittiguna, et al., 1980; Chevillard, et al., 1980; Houchi, et al., 1986; Morita, et al., 1986). Yohimbine is a competitive antagonist that is selective for alpha$_2$-adrenergic receptor; if it enters the CNS, whereas it acts to increase blood pressure and heart rate. These actions are opposite to those of clonidine, an alpha$_2$-adrenergic receptor agonist. Furthermore, yohimbine is also an antagonise of 5-HT (Gerber, et al., 1991). The decrease of blood pressure of rate by yohimbine reported here, may be the result of the recent finding that mammalian alpha adrenoceptors have been identified by molecular cloning and reported 6 types of alpha adrenoceptor. The three alpha$_2$ adrenoceptors share about 75% amino acid identity in their putative transmembrane regions, C.M. Fraser and coworkers (1989) observed that high concentrations of adrenaline (>100 uM) applied to cultured cells expressing alpha$_2$-C$_{10}$-encoded adrenoceptors actually resulted in increased accumulation of cAMP; this effect was also seen with the alpha$_2$adrenoceptor agonists UK 14340 and p-amino-clonidine and was blocked by the antagonist yohimbine (Harrison, 1991). Yohimbine that reduced blood pressure of anesthetized rat reported here might be the blockade of adrenaline and even noradrenaline which caused the alpha$_2$-C$_{10}$-encoded adrenoceptors to produce the accumulation of cAMP. The decreased cAMP might attennuate the vascular tone and thereby the vasodilatation; the fall of blood pressure may be resulted because intraventricular injection of hydralazine produced an hypotensive effect (Gupta and Bhargava, 1965). Yohimbine or phentolamine not hydralazine given intravenously prior to noradrenaline could antagonise the pressor effect of noradrenaline on rat blood pressure. Phentolamine is an imidazole derivative is capable of alpha-adrenceptor blockade lasting for several hours; this blockade is more effective against circulating adrenaline than it is agonist neurally release transmitter (Kroeger, 1985). Phentolamine, however, is a partial agonist and have a sympathomimetic activity and can block the prejunctional alpha$_2$ as well as alpha$_1$adrenoceptor. This will interfere with the negative feedback mechanism that normally limits the amount of noradrenaline released. The neuron fails to shut itself off; and the excess transmitter may produce many sympathetic side effects via unblocked beta adrenoceptors (Stokes and Oates, 1978). It is suggested long time ago, that intravenously administered hydralazine weakened the hypertensive effect of noradrenaline and adrenaline in the cat (Gross. et al., 1950). This is opposite to the results of unability of hydralazne to antagonise rat hypertensive effect of noradrenaline reported here compared with the blockade of noradrenaline induced vasopressor effect by both yohimbine or phentolamine. This may be due to the species difference.

It is suggested, therefore, that hydralazine is not recommended to treat hypertension with hypersecretion of noradrenaline for example pheochromocytoma, neuroblastoma, ganglioneuroma and other catecholamine-producing tumors.

REFERENCES

1. Chevillard C, Mathieu MN, Saiag B, Worcel M. Hydralazine : effect on the outflow of noradrenaline and mechanical responses evoked by sympathetic nerve stimulation of the rat tail artery. British Journal of Pharmacology 1980; 69: 415-420.
2. Gerber JG, and Nies AS. Antihypertensive agents and the drug therapy of hypertension. In : The Pharmacological Basis of Therapeutics. 8 th Goodman AG, Gilman LS, Gilman A, eds. Pergamon Press, Inc. Singagpore 1991: 784-813.
3. Gross F, Druey J, and Meier R. Eine neue Gruppe blutdrucksenkender Substanzen von besonderem Wirkung-Scharakter. Experientia 1950; 6: 19-21.
4. Gupta KP, and Bhargava KP. Mechanism of tachycardia induced by intrancerebroventricular injection of hydralazine (1-hydrazinophthalazine). Archives Internationales de Pharmacodynamie et de. Therapie 1965; 155: 84-89.
5. Harrison JK, Pearson WR, and Lynch RL. Molecular characterization of $_{1}$- and $_{2}$-adrenoceptors. Trends in Pharmacological Sciences 1991; 12 (2): 62-67.
6. Houchi H, Morita K, Minakuchi K, and Oka M. Inhibition by hydralazine of catecholamine biosynthesis in cultured bovine adrenal medullary cells. Biochemical Pharmacology 1986, 35 (12): 2047-2049.
7. Kroeger DC. Adrenergic block drugs. In: Pharmacology and Therapeutics for Dentistry, 2 nd ed. The C.V. Mosby Company, St. Louis, 1985: 121-136.
8. Kruszyma H, Kruszyma R, Smith RP, and Wilcox DE. Red Blood cells generate nitric oxide from directly acting nitrogenous vasodilators. Tozicological and Applied Pharmacology 1987; 91: 429-438.
9. Liu TZ, Shen JT, and Loken HF. inhibition of dopamine-hydroxylase by hydralazine. Proceedings of the Society for Experimental Biology and Medicine 1974; 145: 294-297.
10. Morita K, Houchi H, Nakanishi A, Minakuchi K, and Oka M. Inhibitory action of hydralazine on catecholamine-synthetizing enzymes from bovine adrenal medulla. Japan Journal of Pharmacology, 40: 445-453.
11. Spokas EG, Folco G, Quilley J, Chander P, and McGriff JC. Endothelial mechanism in the vascular action of hydralazine. Hypertension, 5, suppl, I 1983: 1107-1111.
12. Songkittiguna P. Ma jewski H, and Rand MJ. Inhibition by hydralazine of the conversion of dopamine to noradrenaline in rat atria in vitro and in vivo. Clinical and Experimental Pharmacology and Physiology 1980; 7: 509-514.
13. Stokes GS, and Oates HF. Prazosin : new alpha-adrenergic blocking agent in the treatment of hypertension, Cardiovascular Medicine 1978; 3: 41-57.
14. Weiss GB, Winquist RT, and Silver PJ. Vascular smooth muscle and vasodilator. In : Cardiovascualr Pharmacology. 3 rd ed. Edited by M. Antonaccio, Raven Press Ltd. New York, 1990: 75-105.