Cerebrovascular Reactivities to N-Methylated Catecholamines in Humans, Monkeys and Dogs

Hachiro USUI, Hiroaki SHIRAHASE and Kazuyoshi KURAHASHI

Department of Pharmacology, Faculty of Medicine and Pharmacology Division, Radioisotope Research Center, Kyoto University, Kyoto 606, Japan

Accepted on July 2, 1990

Abstract—A comparison was made of the cerebrovascular responses of humans, monkeys and dogs to noradrenaline (NAd) and dopamine (DA) as well as to their N-methylated derivatives, adrenaline (Ad) and N-methyl dopamine (methyl DA). The present experiments demonstrated that N-methylation of both NAd and DA enhanced the contractile responses of these agents in human cerebral arteries, while only N-methylation of DA enhanced the contraction of monkey cerebral arteries, and such an enhancement was not seen in dog cerebral arteries.

Recently, phenylethanolamine-N-methyltransferase activity in cerebral microvessels of the rat has been demonstrated by immunocytochemical and immunohistochemical methods. The demonstration of this enzyme in the extraneuronal compartment and particularly in the cerebrovascular endothelium suggests that the cerebral microvessels are capable of synthesizing adrenaline (Ad) from noradrenaline (NAd) (1). There are few reports on the cerebrovascular reactivities to N-methylated catecholamines. The present experiments were undertaken to compare the response to NAd and dopamine (DA) with those to Ad and N-methyl dopamine (methyl DA) in human, monkey and dog cerebral arteries.

Middle cerebral arteries (1.5 to 3.0-mm outer diameter) were isolated from the brains of 11 humans during autopsy, 3–8 hr after death. The causes of death in the 33- to 77-year-old patients were traffic accidents, stomach cancer, heart failure, stroke, subarachnoid hemorrhage, etc. Japanese monkeys (Macaca fuscata) of either sex, weighing 4–13 kg, were anesthetized with intramuscular injections of ketamin (10 mg/kg) and killed in the same way as the monkeys. The brains were rapidly removed, and the middle cerebral arteries (0.5 to 1.5-mm outer diameter in monkeys; 0.5 to 1.0 mm in dogs) were isolated. These arteries were cut into helical strips, approximately 20-mm long and 1.5 to 2-mm wide (humans), or 1 to 2-mm wide (monkeys and dogs). The rubbing procedure was applied to all preparations. The helical strips were fixed vertically between hooks in an organ bath containing Krebs-Henseleit solution, which was maintained at 37±0.5°C and aerated with a mixture of 95% O2 and 5% CO2. The hook fixing the upper end of the strips was connected to the lever of a force-displacement transducer (T7-8-240, Orientec Co., Tokyo, Japan).

The resting tension was adjusted to 1.5–2.0 g in human arteries and to 1.5 g in monkey and dog arteries. Constituents of the Krebs-Henseleit solution were as follows: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.18 mM MgCl2, 1.18 mM KH2PO4, 25 mM NaHCO3 and 10 mM dextrose. The pH of the solution was 7.3–7.4. The specimens were allowed to equilibrate in the solution at resting tension for a period of 60 min before the experiments were started. During this period, the medium was replaced several times, and the pre-set tension was repeatedly...
adjusted until it remained stable.

Isometric contractions were displayed on an inkwriting oscillograph (NEC San-ei, Tokyo, Japan). Concentration-response curves for NAd, Ad, DA and methyl DA were obtained by adding the agents directly to the bathing media in a cumulative manner.

Statistical analysis was made using Student's t-test for unpaired comparison. Agents used were NAd, Ad, DA and methyl DA, which were all purchased from Nacalai Tesque Co., Kyoto, Japan.

The contractile responses to NAd and Ad were compared in human, monkey and dog cerebral arteries. The addition of NAd and Ad in concentrations ranging from $5 \times 10^{-9}$ to $10^{-5}$ M caused concentration-dependent contractile responses in all preparations obtained from humans, monkeys and dogs (Fig. 1). In human cerebral arteries, the N-methylation of NAd to Ad significantly increased the maximal contraction but not

---

**Fig. 1.** Concentration-response curves for noradrenaline (A) and adrenaline (B) in human, monkey and dog cerebral arteries. Each symbol indicates mean absolute values with S.E. (●): humans, (○): monkeys, (▲): dogs. Figures in parentheses indicate number of preparations.

---

**Fig. 2.** Concentration-response curves for dopamine (A) and methyl dopamine (B) in human, monkey and dog cerebral arteries. Each symbol indicates mean absolute values with S.E. (●): humans, (○): monkeys, (▲): dogs. Figures in parentheses indicate number of preparations.
in monkey and dog cerebral arteries.

The addition of DA and methyl DA in concentrations ranging from $2 \times 10^{-8}$ to $10^{-5}$ M produced a concentration-dependent response, either contraction or relaxation, depending upon the species and preparations (Fig. 2). In human cerebral arteries, DA caused contraction in 7 out of 11 preparations and relaxation in the remaining 4. DA caused relaxation in all preparations obtained from monkeys, but caused contraction in all preparations of dog cerebral arteries. When the responses to DA and methyl DA are compared, it can be seen that methyl DA caused greater contraction than DA in human arteries, and methyl DA caused only a contractile response (Fig. 2). The relaxation produced by DA in monkey cerebral arteries was reversed by N-methylation of DA to a contractile response in about two thirds of the preparations (Fig. 2). In dog cerebral arteries, N-methylation of DA had no significant effect on the contractile response.

The present experiments demonstrated that N-methylation of both NAd and DA enhanced their contractile effects on human cerebral arteries, while only N-methylation of DA enhanced the contraction in monkeys, and no such effect was seen in dogs. Furthermore, N-methylation of DA to methyl DA reduced the number of preparations in which relaxation was produced in both human and monkey cerebral arteries.

Phenylethanolamine - N - methyltransferase activity in rat cerebral microvessels has recently been demonstrated using immunocytochemical and immunohistochemical methods. This enzyme activity in the vessels was found in the endothelium. Both circulating NAd and NAd released from nerve terminals in the blood vessels may be converted to Ad in cerebral vessels (1). Indeed, inhibitors of phenylethanolamine-N-methyltransferase decreased the systemic blood pressure of the rat (2–4). The contractile response to N-methylated catecholamines (Ad and methyl DA) of cerebrovascular preparations, higher than those of NAd and DA, may play an important role in cerebrovascular tone.

Since the contractile response of cerebral arteries to NAd is quite weak and resistant to phentolamine and phenoxybenzamine, it has been postulated that alpha adrenergic receptors in dog, cat, and rabbit cerebral arteries are different in nature from those in extracerebral arteries (5–10). In previous studies using ligand binding experiments, we reported that human and monkey cerebral arteries contained both alpha 1 and 2 adrenergic receptors, but dog cerebral arteries contained only alpha 2 adrenergic receptors (11, 12). Although in the present studies, we did not determine if N-methylation of the catecholamines results in higher affinity of catecholamines to alpha 1 receptors alone, this would explain the enhanced response to Ad and methyl DA in human and monkey cerebral arteries and the absence of any change in responsiveness in dog cerebral arteries lacking alpha 1 receptors.

Taken together with our previous binding studies on distribution of alpha 1 and 2 adrenergic receptors in these preparations (12), the present studies suggest that the increase in contractile response to N-methylated catecholamines may relate to the distribution of alpha 1 adrenergic receptors in human and monkey cerebral arteries. Since dog cerebral arteries lack alpha 1 receptors, N-methylation would not enhance the effects of catecholamines.

Acknowledgments: This study was supported in part by Grant in Aids for Developmental Research (60870010) and Special Project Research (61232007) and (62222010) from the Ministry of Education, Science and Culture, Japan, and the Smoking Research Foundation, Japan.

References
1 Spatz, M., Nagatsu, I., Maruki, C., Yoshida, M., Kondo, Y. and Bembry, J.: The presence of phenylethanolamine-N-methyltransferase in cerebral microvessels and endothelial cells. Brain Res. 240, 191–194 (1982)
2 Saavedra, J.M., Grobecker, H. and Axelrod, J.: Adrenaline-forming enzyme in brainstem: elevation in genetic and experimental hypertension. Science 191, 483–484 (1976)
3 Saavedra, J.M.: Adrenaline levels in brain stem nuclei and effects of a PNMT inhibitor on spontaneously hypertensive rats. Brain. Res. 166, 283–292 (1979)
4 Goldstein, M., Kinguasa, K., Hieble, J.P. and Pendleton, R.G.: Lowering of blood pressure in
hypertensive rats by SKF 64139 and SKF 72223. Life. Sci. 30, 1951–1957 (1982)

5 Toda, N. and Fujita, Y.: Responsiveness of isolated cerebral and peripheral arteries to serotonin, norepinephrine, and transmural electrical stimulation. Circ. Res. 33, 98–104 (1973)

6 Edvinsson, L. and Owman, C.: Pharmacological characterization of adrenergic alpha and beta receptors mediating the vasomotor responses of cerebral arteries in vitro. Circ. Res. 35, 835–849 (1974)

7 Toda, N., Hayashi, S. and Hattori, K.: Analysis of the effect of tyramine and norepinephrine in isolated canine cerebral and mesenteric arteries. J. Pharmacol. Exp. Ther. 205, 382–391 (1978)

8 Duckles, S.P. and Bevan, J.A.: Pharmacological characterization of adrenergic receptors of a rabbit cerebral artery in vitro. J. Pharmacol. Exp. Ther. 197, 371–378 (1976)

9 Sakakibara, Y., Fujiwara, M. and Muramatsu, I.: Pharmacological characterization of the alpha adrenoceptors of the dog basilar artery. Naunyn Schmiedebergs Arch. Pharmacol. 391, 1–7 (1982)

10 Shibata, S.: The effects of drugs on the autonomic neuroeffector system of cerebral arteries. In: Factors Influencing Vascular Reactivity, Edited by Carrier, O. and Shibata, S., p. 132–155, Igaku-Shoin, Tokyo, New York (1977)

11 Tsukahara, T., Taniguchi, T., Fujiwara, M. and Handa, H.: Characterization of alpha adrenoceptors in pial arteries of bovine brain. Naunyn Schmiedebergs Arch. Pharmacol. 324, 88–93 (1983)

12 Usui, H., Fujiwara, M., Tsukahara, T., Taniguchi, T. and Kurahashi, K.: Differences in contractile responses to electrical stimulation and α-adrenergic binding sites in isolated cerebral arteries of human, cows, dogs and monkeys. J. Cardiovasc. Pharmacol. 7, Supp. 3, s47–s52 (1985)