Changes in Plasma Catecholamine Levels following Injection of Prostaglandin $F_{2\alpha}$ into the Basal Cistern in Rabbits

Yae YOKOYAMA, Morio UCHIDA, Sanae MATSUMOTO, Keizo SAITO and Makoto FUKUDA

We measured plasma epinephrine and norepinephrine concentrations in a rabbit model simulating subarachnoid hemorrhage (SAH), following the injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) into the basal cistern. In this model, plasma epinephrine values increased significantly (to 4.2-fold those before injection), substantially more than norepinephrine (which increased 1.3-fold) at 5 minutes (min) after $PGF_{2\alpha}$ injection. Dissection of autonomic outflow from the cervical spinal cord or ligation of the suprarenal veins reduced the changes in plasma catecholamine concentrations associated with $PGF_{2\alpha}$ injection. These results suggest that the sympathetic discharge seen after $PGF_{2\alpha}$ injection into the basal cistern in rabbits occurred through the sympatho-adrenal pathways. (Key words: plasma epinephrine, plasma norepinephrine, subarachnoid hemorrhage, prostaglandin $F_{2\alpha}$, rabbits)

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Electrocardiographic (ECG) abnormalities are frequently seen in association with cerebro-vascular disorders[1]. Also, in patients undergoing cerebral aneurysm clipping, sudden ST-T changes and cardiac arrhythmias sometimes occur[2,3]. These changes, including focal myocytolysis and subendocardial damage, appear to be caused by hypothalamic stimulation leading to an acute increase in sympathetic tone[4,5]. Urinary[6] and plasma[7,8] catecholamines are elevated in patients and in experimental animal models[9–12] with SAH. The effects of SAH on the heart are thought to be related to a massive release of catecholamine, and to stimulation of the sympathetic cardiac nerve[1] or the sympathoadrenal system[7]. In 1989, we reported that the intracisternal injection of 0.5 ml of 0.1% prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in rabbits produced severe ECG changes resembling those seen in patients with SAH[13]. In this study, in our first experiment, to elucidate whether massive sympathetic discharge was produced[14], we measured plasma catecholamine levels in these rabbit models. In the second experiment, we examined the effects of pretreatment, by either spinal sympathectomy at the C2 level or by ligation of the suprarenal veins, on plasma catecholamine levels.

Department of Anesthesiology, Kansai Medical University, Osaka, Japan

Address reprint requests to Dr. Yokoyama: Department of Anesthesiology, Kansai Medical University, 1 Fumizono-cho, Moriguchi, Osaka, 570 Japan

Materials and Methods

Forty-two domestic rabbits weighing 2.0–2.5 kg were used. The method of approach to the basal cistern has been described previously[13]. Briefly, each rabbit was anesthetized with di-ethyl ether and fixed in the supine position, a tracheostomy was performed, and ventilation was maintained with a Harvard small animal respirator (South Natick, Massachusetts). Muscle relaxation was obtained using pancuronium bromide (0.1 $mg \cdot kg^{-1}$). A venous line was placed in an ear vessel and an arterial line was placed in the femoral artery. The retropharyngeal mucous membrane was ablated rostrally until the basisphenoid bone became visible. The orifice of the craniopharyngeal duct was then exposed on the surface of the basisphenoid bone. A needle was inserted through the orifice and at the depth of 6 mm, the chiasmatic cistern was reached. A teflon catheter (1.0 mm outside diameter) was placed over it and approximately 0.3 ml of cerebrospinal fluid was withdrawn. After 15 min, 0.5 ml of 0.1% $PGF_{2\alpha}$ or physiological saline was injected through the catheter into the chiasmatic cistern. The experiments were carried out in two stages.

First experiment: In twelve rabbits, 0.5 ml of physiological saline was injected into the chiasmatic cistern. In fourteen other rabbits, 0.5 ml of 0.1% $PGF_{2\alpha}$ (in 25 mM sodium acetate solution) was injected into the chiasmatic cistern. For the determination of catecholamine levels, blood samples were taken from the femoral artery just before and 5 min after the intracisternal injection of drugs.

Second experiment: In six rabbits, the spinal cord was dissected at a high cervical level (C2) to obtain complete denervation to both cardiac sympathetic nerve and adrenal medulla (C2-dissected group). In five rabbits ligations of the suprarenal veins from bilateral adrenal glands were performed following abdominal incision (suprarenal veins ligated group). As a control group, another five rabbits were left untreated (untreated group). Blood was sampled in all groups 15 min after the end of the procedure aimed at the elimination of circulatory reflexes. A 0.5-ml of 0.1% $PGF_{2\alpha}$ was then injected into the chiasmatic cistern, as in the first experiment. Plasma catecholamine levels were measured just before and at 3, 5 and 10 min after injection.

Assay of plasma catecholamines: A 2-ml of arterial blood sample was taken and immediately centrifuged at 3000 rpm for 15 min at 4°C. The supernatant plasma was stored at −20°C. Measurements were made within two weeks. One hundred milligrams of activated alminum oxide was mixed with 1 ml of dissolved plasma and 1 M-Tris HCl buffer (pH 8.6). The mixture was shaken for 15 min and washed out with 15 ml of pure water; the catecholamines were finally extracted using 0.3 ml of 0.2 M perchloric acid. The samples were separated and quantitated using high performance liquid chromatography (Shimadzu shim-pack CLC-ODS) coupled with spectrofluorometry. Catecholamine concentrations were determined using a trihydroxyindole method[15]. We were able to obtain 75% recovery of norepinephrine and 78% recovery of epinephrine (mean values). Intra-assay sensitivity was 0.025 $ng \cdot ml^{-1}$ plasma. Data are presented as mean values ± SEM $ng \cdot ml^{-1}$ plasma.

We compared conditions before and after injection using grouped Student's *t* test for the first experiment and analysis of variance for the second experiment. The significance of changes in a group was evaluated using Student's *t* test for paired samples. Differences were considered significant when $P <$

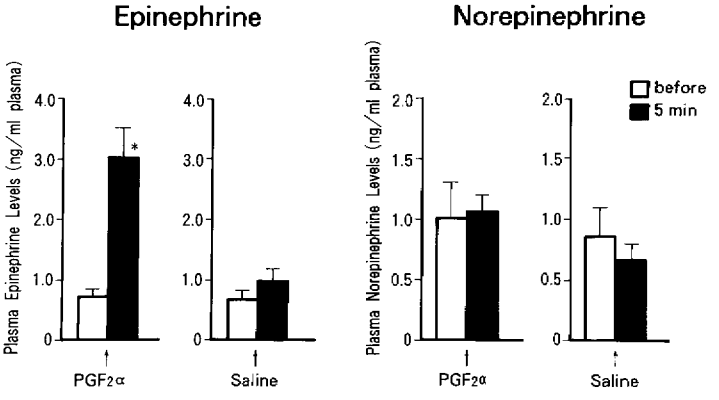

Fig. 1. Changes in plasma epinephrine and norepinephrine following the injection of normal saline or $PGF_{2\alpha}$ into the chiasmatic cistern of rabbits.

Values are expressed as mean ± SEM $ng \cdot ml^{-1}$ plasma

*: $P < 0.05$ vs before injection

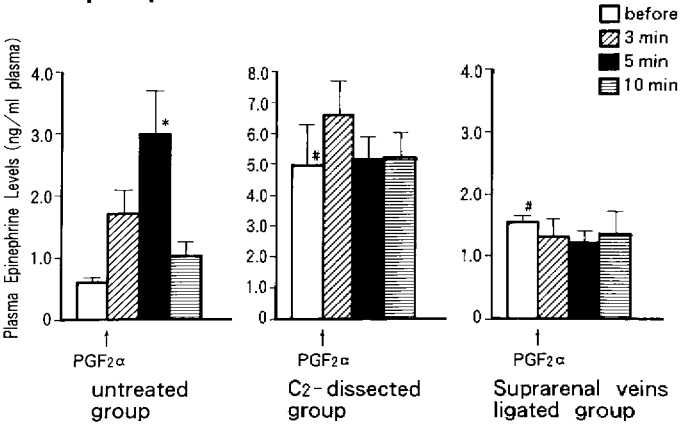

Fig. 2. Time course changes in plasma epinephrine in untreated (n=5), C2-dissected (n=6) and suprarenal veins ligated (n=5) groups following the injection of $PGF_{2\alpha}$ into the chiasmatic cistern.

Values are expressed as mean ± SEM $ng \cdot ml^{-1}$ plasma

*: $P < 0.05$ vs before injection

#: $P < 0.05$ vs before injection in untreated group

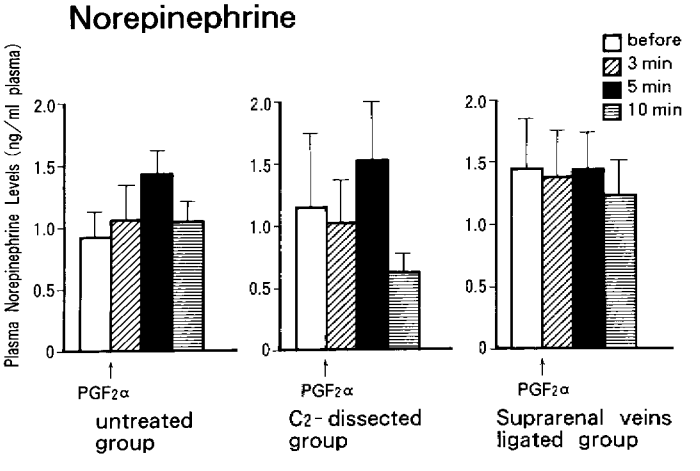

Fig. 3. Time course changes in plasma norepinephrine in untreated (n=5), C2-dissected (n=6) and suprarenal veins ligated (n=5) groups following the injection of $PGF_{2\alpha}$ into the chiasmatic cistern.

Values are expressed as mean ± SEM $ng \cdot ml^{-1}$ plasma

P = Not significant

0.05.

Results

In the first experiment, we observed the values for plasma epinephrine and norepinephrine before and 5 min after injection of normal saline or $PGF_{2\alpha}$ into the chiasmatic cistern (fig. 1). Before injection there were no differences between the two groups in

either epinephrine or norepinephrine levels. As shown in figure 1, plasma epinephrine concentrations increased significantly (from 0.72 ± 0.13 to 3.04 ± 0.56 ng·ml^{-1}, $P < 0.05$) after $PGF_{2\alpha}$ injection in contrast to values after normal saline injection. There were, however, no significant changes in plasma norepinephrine levels at pre-injection and at 5 min after the injection of either $PGF_{2\alpha}$ or normal saline. ECG abnormalities were observed at 121.8 ± 103.9 seconds (Mean ± SD) following the injection of 0.5 ml of 0.1% $PGF_{2\alpha}$ in the untreated rabbits (submitted for publication). Time course changes in plasma epinephrine and norepinephrine levels are shown in figures 2 and 3, respectively. In the untreated group, plasma epinephrine levels at 5 min after the injection of $PGF_{2\alpha}$ were significantly higher than those before injection ($P < 0.05$); this finding was similar to that in the first experiment. In the C2-dissected and the suprarenal veins ligated groups, plasma epinephrine levels before the injection of $PGF_{2\alpha}$ were significantly higher than those in the untreated group ($P < 0.05$). On the other hand, the increase in plasma epinephrine following the injection of $PGF_{2\alpha}$ was not significant in the C2-dissected group, and a rather decreasing tendency in plasma epinephrine was observed in the suprarenal veins ligated group. Plasma norepinephrine levels at 3, 5 and 10 min after the injection of $PGF_{2\alpha}$ did not differ significantly from those before injection in any of the three groups.

Discussion

In the first experiment, plasma epinephrine levels were markedly elevated by intracisternal $PGF_{2\alpha}$, but the increase in plasma norepinephrine was only slight. This result showed that a sympathetic discharge occurred in this rabbit model following the injection of $PGF_{2\alpha}$ into the chiasmatic cistern. It is known that 75%–80% of the norepinephrine released from sympathetic nerve endings is immediately either taken up again and inactivated at the nerve ending or metabolized; only a small portion of the norepinephrine is released into the circulation[16]. Majewski et al.[17] found that a high dose of epinephrine infusion in pithed rabbits with electrically stimulated sympathetic outflow reduced the rate of release of norepinephrine, due to activation of the inhibitory presynaptic α-adrenoceptor system. In our study also, no significant elevation in plasma norepinephrine concentrations was observed in any group.

Arrhythmias resembling SAH, which occurred after 3 to 5 min following injection of $PGF_{2\alpha}$ in our previous study[13], were synchronized with the maximum plasma epinephrine and norepinephrine concentrations in this study.

The dose of 0.5 ml of physiological saline did not increase plasma catecholamine levels significantly. It is considered that the increase of epinephrine by $PGF_{2\alpha}$ has been attributed not to the elevation of intracranial pressure, but to the properties of $PGF_{2\alpha}$ itself. Motomochi[9] reported that plasma epinephrine increased to a maximum level 10 seconds after the injection of 0.75 ml·kg^{-1} of blood into the chiasmatic cistern in cats. Boddin et al.[10] reported that maximum plasma epinephrine levels occurred at 45 seconds after the injection of 10–12 ml of blood into the fossa cerebralis posterior in dogs. This earlier release of epinephrine, compared with our result, would reflect the elevation of intracranial pressure due to the high dose of blood[18,19].

Locally applied $PGF_{2\alpha}$ produces cerebral vasospasm[20], although the mechanism resulted in tachycardiac responses by the intracerebroventric-

ular administration of $PGF_{2\alpha}$ may include direct neuronal stimulation[21]. Peak plasma catecholamine concentrations are observed in the development of cerebral vasospasm following clipping operations[22]. $PGF_{2\alpha}$ would stimulate the sympathetic nervous center in the hypothalamus by introducing local cerebral vasospasm in this model.

Various nonphysiological stimuli, e.g., anesthetics such as di-ethyl ether[23], operating procedures, and hemorrhage can lead to an increase in epinephrine and norepinephrine. In the C2-dissected group, especially, plasma epinephrine levels were much higher than those in the untreated group. It seems likely that injury of the high cervical spine strongly influenced plasma catecholamine levels[24,25]. In the suprarenal veins ligated group, variable stress, e.g., pain due to the procedure of incising the abdominal area under light anesthesia, bleeding, and touching the adrenal glands may have influenced the preinjection values[26]. Garbulinski et al.[27] have shown that epinephrine and norepinephrine values at 15 min after adrenalectomy in rabbits anesthetized with urethane were 1.34 ± 0.38 ng·ml^{-1} and 1.92 ± 0.78 ng·ml^{-1}, respectively. Our data were comparable with theirs.

In the second experiment, dissection of the cervical spinal cord (C2) inhibited the rise of plasma catecholamines. The changes in plasma epinephrine do not seem to be a ceiling effect, since far higher plasma epinephrine concentrations have been found after head injury[28]. The increase of plasma epinephrine and norepinephrine was abolished by the pretreatment of ligation of the suprarenal veins. The high percentage of epinephrine in the adrenal catecholamines of rabbits (96% epinephrine, 4% norepinephrine[29]) compared to that in cats, dogs and men, means that almost all the circulating epinephrine came from the adrenal medulla[30]. These findings showed that the descending sympatho-adrenomedullary pathways could be cut off by the dissection at the C2 level, and massive catecholamine release was abolished by the ligation of the suprarenal veins.

In conclusion, these results suggest that the massive sympathetic discharge seen after $PGF_{2\alpha}$ injection into the basal cistern occurs through the sympatho-adrenomedullary pathways.

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References

1. Stober T, Kunze K: Electrocardiographic alternations in subarachnoid hemorrhage. J Neurology 227:99–113, 1982
2. Samra SK, Kroll DA: Subarachnoid hemorrhage and intraoperative electrocardiographic changes simulating myocardial ischemia-anesthesiologist's dilemma. Anesth Analg 64:86–89, 1985
3. Tanaka M, Dohi S: Anesthetic management of a patient with severe subarachnoid hemorrhage and diffuse lung edema. Masui (Jpn J Anesthesiol) 38:1083–1091, 1989
4. Marion DW, Segal R, Thompson ME: Subarachnoid hemorrhage and the heart. Neurosurg 18:101–106, 1986
5. Sugiura M, Yozawa Y, Kubo O, et al: Myocardial damage-myocytolysis-caused by subarachnoid hemorrhage. Noshinkei 37:1155–1161, 1985
6. Neil-Dwyer G, Cruikshank J, Stott A, et al: The urinaly catecholamine and plasma cortisol levels in patients with subarachnoid hemorrhage. J Neurol Sci 22:375–382, 1974
7. Benedict CR, Loach AB: Sympathetic nervous system activity in patients with subarachnoid hemorrhage. Stroke 9:237–244, 1978
8. Emanuele MA, Dorsch TR, Scarff TB, et al: Basilar artery aneurysm simulating pheochromocytoma. Neurology 31:1560–1561, 1981
9. Motomochi M: Arrhythmias in subarachnoid hemorrhage model in cat:

Role of the anterior hypothalamic area. Arch Jpn Chir 54:172–188, 1985
10. Boddin M, Bogaert AV, Dierick W: Catecholamines in blood and myocardial tissue in experimental subarachnoid hemorrhage. Cardiology 58:229–237, 1973
11. Lacombe PM, Iadecola C, Underwood MD, et al: Plasma epinephrine modulates the cerebrovasodilation evoked by electrical stimulation of dorsal medulla. Brain Research 506:93–100, 1990
12. Stoddard-Apter SL, Siegel A, Levin BE: Plasma catecholamine and cardiovascular responses following hypothalamic stimulation in the awake cat. J Auto Nerv Sys 8:343–360, 1983
13. Uchida M, Saito K, Niitsu T, et al: Model of electro-cardiographic changes seen with subarachnoid hemorrhage in rabbits. Stroke 20:112–118, 1989
14. Redgate ES, Gellhorn E: Nature of sympathetico-adrenal discharge under conditions of excitation of central autonomic structures. Am J Physiol 174:475–480, 1953
15. Anton AH, Sayre DF: A study of factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J Pharmacol Exp Ther 138:360–375, 1962
16. Iversen LL: Catecholamine uptake processes. Br Med Bull 29:130–135, 1973
17. Majewski H, Schurr LHC, Starke K: Dual effect of adrenaline on noradrenaline release in the pithed rabbit. J Cardiovasc pharmacol 7:251–257, 1985
18. Graf CJ, Rossi NP: Catecholamine response to intracranial hypertension. J Neurosurg 49:862–868, 1978
19. Norris JW: Cardiac effects of acute cerebrovascular lesions, Neurocardiology. Edited by Kulbertus HE, Franck G. New York, Futura Pub Com, 1988, pp. 211–219
20. Uski TK, Andersson KE, Brandt L, et al: Characterization of the prostanoid receptors and of the contractile effects of prostaglandin $F_{2\alpha}$ in human pial arteries. Acta Physiol Scand 121:369–378, 1984
21. Rao TS, Seth SD, Manchanda SC, et al: The involvement of the sympathetic nervous system in the centrogenic pressor and tachycardiac effects of prostaglandins E_2 and $F_{2\alpha}$ in anaesthetised cats. Brain Research 435:7–14, 1987
22. Loach AB, Benedict CR: Plasma catecholamine concentrations associated with cerebral vasospasm. J Neurol Sci 45:261–271, 1980
23. Inoue M, Kurahashi K, Fujiwara M: Noradrenaline turn over after left coronary artery ligation in rat heart. European J Pharmacol 143:251–257, 1987
24. Buhler HU, Prada MDA, Haefely W, et al: Plasma adrenaline, noradrenaline and dopamine in man and different animal species. J Physiol 276:311–320, 1978
25. Hart BB, Stanford GG, Ziegler MG, et al: Catecholamines: Study of interspecies variation. Crit Care Med 17:1203–1222, 1989
26. Guyton AC: The autonomic nervous system; the adrenal medulla, The Textbook Medical Physiology. Eighth Edition. Edited by Guyton AC. Philadelphia, WB Saunders, 1991, pp. 667–678
27. Garbulinski T, Switala M: Endogenous catecholamines during noradrenaline and adrenaline hypertension. Pol J Pharmacol Pharm 31:89–96, 1979
28. Beckman DL, Iams SG: Circulating catecholamines in cats before and after lethal head injury. Proc Soc Exp Biol Med 160:200–202, 1979
29. Schadt JC, Gaddis RR: Endogenous opiate peptides may limit norepinephrine release during hemorrhage. J Pharmacol Exp Ther 232:656–660, 1985
30. Fillenz M: Catecholamines and sympathetic innervation, Cardiac Metabolism. Edited by Drake-Holland AJ, Noble MIM. New York, John Wile and Sons Ltd, 1983, pp. 471–486