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Effects of *dl*-3-*n*-butylphthalide on production of TXB_2 and 6-keto-$PGF_{1\alpha}$ in rat brain during focal cerebral ischemia and reperfusion[1]

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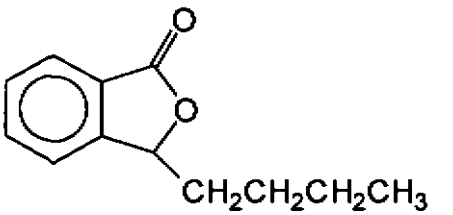
KEY WORDS *dl*-3-*n*-butylphthalide; thromboxane B_2; 6-ketoprostaglandin F_1 alpha; aspirin; transient cerebral ischemia

AIM: To study the effects of *dl*-3-*n*-butylphthalide (NBP) on the changes of thromboxane B_2 (TXB_2) and 6-keto-PGF_1 alpha (6-keto-$PGF_{1\alpha}$) contents in hippocampus, striatum, and cerebral cortex of rats subjected to focal cerebral ischemia followed by reperfusion. **METHODS**: Focal cerebral ischemia was induced by inserting a nylon suture into intracranial segment of internal carotid artery from external carotid artery and blockade of the origin of middle cerebral artery. For reperfusion, the suture was pulled out to restore the blood flow to the ischemic brain. Determination of TXB_2 and 6-keto-$PGF_{1\alpha}$ was performed by RIA method. **RESULTS**: Reperfusion following focal cerebral ischemia resulted in increases in TXB_2 at 5 min and 6-keto-$PGF_{1\alpha}$ at 30 min and a decrease in the ratio of epoprostenol (PGI_2)/thromboxane A_2 (TXA_2) (6-keto-$PGF_{1\alpha}$/TXB_2) at 5 min in hippocampus, striatum, and cerebral cortex. NBP 10 $mg \cdot kg^{-1}$ reduced the content of TXB_2 without decreasing effect on 6-keto-$PGF_{1\alpha}$. NBP 20 $mg \cdot kg^{-1}$ reduced both TXB_2 and 6-keto-$PGF_{1\alpha}$ in lesser extent than aspirin (Asp, 20 $mg \cdot kg^{-1}$). NBP 20 or 10 $mg \cdot kg^{-1}$ elevated the ratio of PGI_2/TXA_2 after reperfusion, but Asp 20 $mg \cdot kg^{-1}$ did not increase the ratio except in striatum at 5 min after reperfusion. **CONCLUSION**: NBP increases the ratio of PGI_2/TXA_2 which may have beneficial effects on the impaired microcirculation in postischemic brain tissues.

In previous studies, we found that *dl*-3-*n*-butylphthalide (NBP) possessed protective effects against cerebral ischemia insult[1-4]. NBP improved ischemic brain energy metabolism in mice[1], reduced the infarct size after middle cerebral artery occlusion (MCAO) in rats[2], and attenuated delayed neuronal damage after cerebral ischemia[3]. The metabolites of AA might contribute to the pathogenic consequences of brain ischemia[4,5]. The cyclooxygenase product of AA, epoprostenol (PGI_2), is a potent vasodilator and platelet aggregation inhibitor, but thromboxane A_2 (TXA_2) has contrary effects on blood vessel and platelet. In this study, we examined the contents of TXB_2 and 6-keto-$PGF_{1\alpha}$, the stable metabolites of PGI_2 and TXA_2, in discrete brain regions after focal cerebral ischemia and reperfusion, attempting to evaluate the effects of NBP on the changes of postischemic production of the metabolites of AA.



***dl*-3-*n*-Butylphthalide**

MATERIALS AND METHODS

Agents *dl*-3-*n*-Butylphthalide (NBP), synthesized by Department of Medicinal Synthetic Chemistry of our Institute, purity > 96 %, was made into emulsion with Tween 80. ^{125}I-thromboxane B_2 and ^{125}I-6-keto-$PGF_{1\alpha}$ RIA kits were purchased from General Hospital of PLA, Beijing.

Temporary focal cerebral ischemia model Wistar rats, weighing 450 ± *s* 46 g, were anesthetized with 10% chloral hydrate (400 $mg \cdot kg^{-1}$, ip). Under an operating microscope, the right common carotid artery (CCA) and its branches, the external (ECA) and internal carotid artery (ICA), were separated. After the branches of ECA were coagulated, a silicon coated monofilament nylon suture (ϕ 0.3 mm) was inserted into ECA lumen. Then the suture was introduced into intracranial segment of ICA through extracranial part of ICA. Approximately 20 mm of the suture (from the origin of ICA)

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being inserted into ICA, resistance was felt, indicating that the tip of the suture had been passed the middle cerebral artery (MCA) origin and reached the proximal segment of the anterior cerebral artery. After 170 min of occlusion, the physical signs of rats were observed. The rats which were unable to display any ischemic signs (failure to extend left fore paw fully; circling to the left and being difficult to turn right) were discarded. Ten min later, the nylon suture was pulled out and the stump of ECA was closed to let the blood restore into the brain.

Experimental groups The experiment was carried out in 5 groups: (A) Sham group received operation but without insertion of nylon suture, $n = 5$; (B) Vehicle group received 0.5 % Tween 80 1 mL·kg^{-1} ip at 5 min and 1 h after the onset of ischemia; (C) NBP 10 mg·kg^{-1} group; (D) NBP 20 mg·kg^{-1} group; (E) Aspirin (Asp) 20 mg·kg^{-1} group. The drugs in group C – E were injected ip at the same times as group B and 7 animals were used at each time point in groups B – E.

Determination of TXB_2 and 6-keto-$PGF_{1\alpha}$ Rats were decapitated at 0, 5, 30 min and 3 h after reperfusion. The forebrain was removed. Then the right hippocampus, striatum, and cerebral cortex were rapidly separated from right hemisphere. About 20 mg of discrete tissues were homogenized with cold ethanol 0.1 mL and normal saline 0.9 mL. The homogenates were spun at 2054 × g for 15 min. The contents of TXB_2 and 6-keto-$PGF_{1\alpha}$ in supernatants were determined according to instructions of RIA kits.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and analyzed with t-test between groups. 6-keto-$PGF_{1\alpha}$/TXB_2 were expressed as PGI_2/TXA_2.

RESULTS

TXB_2 A great increase of TXB_2 in hippocampus, striatum, and cerebral cortex in vehicle group at 5 min ($P < 0.01$ compared with the same group before reperfusion), a subsequent decline at 30 min, and a slightly increase at 3 h (not significantly) after reperfusion were observed. NBP significantly reduced the production of TXB_2 in hippocampus and striatum at 5 min and reduced TXB_2 content in cerebral cortex at 3 h after reperfusion. Asp 20 mg·kg^{-1} reduced TXB_2 in all 3 brain regions at 5 min or decreased TXB_2 in cerebral cortex and hippocampus at 3 h after reperfusion (Tab 1).

6-keto-$PGF_{1\alpha}$ The content of 6-keto-$PGF_{1\alpha}$ in hippocampus, striatum and cerebral cortex increased at 30 min after reperfusion following 3-h focal cerebral ischemia in vehicle group. NBP 20 mg·kg^{-1} reduced the elevation of 6-keto-$PGF_{1\alpha}$ at 30 min after reperfusion ($P < 0.05$) in all discrete tissues, but NBP 10 mg·kg^{-1} showed no such effect. Asp 20 mg·kg^{-1} decreased the level of 6-keto-$PGF_{1\alpha}$ in all 3 discrete brain tissues 30 min and 3 h after reperfusion (Tab 1).

PGI_2/TXA_2 A 3-h focal cerebral ischemia did not change the ratio of PGI_2/TXA_2, but 5-min reperfusion caused a decrease in PGI_2/TXA_2 in hippocampus, striatum, and cerebral cortex in vehicle group. NBP 10 mg·kg^{-1} increased the level of the ratio in the three discrete brain regions at 5 min, in hippocampus and striatum at 30 min, and in cerebral cortex at 3 h after reperfusion. NBP 20 mg·kg^{-1} also elevated the ratio in striatum and cerebral cortex at 5 min, in hippocampus at 30 min and in striatum at 3 h after reperfusion. Asp decreased the ratio in hippocampus ($P < 0.05$), striatum, and cerebral cortex ($P > 0.05$) before reperfusion and reduced the level of PGI_2/TXA_2 in cerebral cortex 30 min after reperfusion. The elevating effect of Asp on the ratio was seen only in striatum 5 min after reperfusion (Tab 1).

DISCUSSION

Our study indicated that the content of 6-keto-$PGF_{1\alpha}$ and TXB_2 in brain regions increased significantly at different stage after focal cerebral ischemia followed by reperfusion and that the ratio of PGI_2/TXA_2 decreased to a very low level at 5 min after reperfusion, may contribute to ischemia/reperfusion injury of brain. The results were consistent with previous report[4]. Our results also showed that NBP significantly reduced the postischemic production of TXB_2 in brain. NBP (10 mg·kg^{-1}) showed no decreasing effect on 6-keto-$PGF_{1\alpha}$. NBP (10 and 20 mg·kg^{-1}) increased the ratio of PGI_2/TXA_2 at most time points, though it reduced the ischemic brain content of 6-keto-$PGF_{1\alpha}$ at the dosage of 20 mg·kg^{-1} at 5 min after reperfusion. Compared with NBP (20 mg·kg^{-1}, Asp seemed to inhibit 6-keto-$PGF_{1\alpha}$ in a more severe extent and for a longer time and failed to improve the ratio of PGI_2/TXA_2 after ischemia and reperfusion. In this point, NBP may have an Asp-like effect on the PGI_2/TXA_2 and may be a more prospective drug than Asp in the treatment of cerebral ischemia.

Tab 1. Effects of NBP and Asp on the changes of TXB_2, 6-keto-$PGF_{1\alpha}$, and PGI_2/TXA_2 in hippocampus, striatum, and cerebral cortex of rats subjected to focal cerebral ischemia and reperfusion. Values are expressed as ng/g wet tissue. $n = 5-7$, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* vehicle (0 min); $^eP < 0.05$, $^fP < 0.01$ *vs* vehicle of corresponding time.

Reperfusion time	Hippocampus				Corpus striatum				Cerebral cortex			
	0 min	5 min	30 min	3 h	0 min	5 min	30 min	3 h	0 min	5 min	30 min	3 h
TXB_2												
Sham	4.1±3.7				3.3±1.6				5.4±5.1			
Vehicle	9.4±7.4	42.5±17.3^c	6.6±4.2	15.4±5.9	3.4±1.2	24.2±9.8^c	3.7±3.1	8.5±6.4	4.8±4.4	18.6±7.1^e	4.1±3.8	13.1±6.4
NBP (mg·kg^{-1})												
10	9.6±7.1	15.9±5.7^f	4.2±3.0	8.3±5.2	3.9±2.7	8.2±2.5^e	5.1±3.4	4.4±3.3	3.8±2.9	15.1±8.8	3.0±2.6	4.4±3.3^e
20	7.5±6.0	17.8±13.5^e	5.5±4.4	8.9±3.5	3.2±2.6	11.4±5.2^e	3.3±2.7	6.2±5.2	5.1±5.0	13.9±5.8	2.4±2.1	6.2±5.2^e
ASP (mg·kg^{-1})												
20	9.8±6.3	16.4±5.6^f	6.1±2.9	6.8±5.4^e	5.0±1.6	4.9±2.9^f	2.4±2.4	4.1±3.8	4.8±3.6	3.5±1.8^f	3.6±3.1	4.1±3.8^e
6-keto-$PGF_{1\alpha}$												
Sham	19±10				16±8				20±10			
Vehicle	25±6	26±10	44±14^b	47±25	14±5	21±8	24±9^b	24±2^c	15±3	18±7	32±14^b	43±27^b
NBP (mg·kg^{-1})												
10	28±12	25±17	39±19	38±33	15±6	18±5	21±4	16±13	18±6	17±7	28±14	38±30
20	26±16	19±6	24±13^f	37±30	12±6	17±9	13±4^f	20±7	18±12	19±13	15±7^f	22±7
ASP (mg·kg^{-1})												
20	18±7	23±14	23±3^f	17±8^f	12±11	17±12	8±6^f	11±9^f	9±3^f	14±7	13±6^f	15±12^f
PGI_2/TXA_2												
Sham	4.9±2.0				5.0±2.3				5.9±3.5			
Vehicle	4.6±2.6	0.8±0.4^c	6.4±1.8	3.4±2.1	4.1±2.0	0.9±0.2^c	8.1±2.9	3.0±1.1	7.5±4.5	1.3±0.2	7.9±1.4	3.1±1.9
NBP (mg·kg^{-1})												
10	3.5±2.3	1.9±0.3^e	13.0±4.9^e	8.9±4.6	5.6±0.9	2.1±0.9^e	13.5±3.4^e	4.3±1.3	5.1±3.3	7.8±0.9^f	10.9±7.0	8.9±4.6^e
20	4.2±2.8	0.9±0.3	10.3±3.9^e	5.1±3.1	5.1±2.6	1.7±0.6^e	10.7±3.9	5.4±2.2^e	8.0±6.0	9.3±0.6^f	7.1±4.0	4.0±2.0
ASP (mg·kg^{-1})												
20	1.8±0.6	1.6±0.9^e	7.9±4.1	2.9±1.2	2.8±2.7	3.7±1.7^f	4.5±2.0	4.3±2.0	2.2±0.9	2.7±1.7	4.0±1.7^e	2.9±1.7

REFERENCES

1 Feng YP, Hu D, Zhang LY. Effect of *dl*-butylphthalide (NBP) on mouse brain energy metabolism in complete brain ischemia induced by decapitation. Acta Pharm Sin 1995; **30**: 741–4.

2 Liu XG, Feng YP. Protecve effect of *dl*-3-*n*-butylphthalide on ischemic neurological damage and abnormal behavior in rats subjected to focal ischemia. Acta Pharm Sin 1995; **30**: 896–903.

3 Lin JF, Feng YP. Effect of *dl*-3-*n*-butylphthalide on delayed neuronal damage after focal cerebral ischemia and intrasynaptosomes calcium in rats. Acta Pharm Sin 1996; **31**: 166–70.

4 Kempski O, Shohami E, von Lubitz D, Hallenbeck JM, Feuerstein G. Postischemic production of eicosanoids in gerbil brain. Stroke 1987; **18**: 111–9.

5 Tegtmeier F, Weber C, Heister U, Haker I, Scheller D, Nikolov R, *et al*. Eicosanoids in rat brain during ischemia and reperfusion — correlation to DC depolarization. J Cerebral Blood Flow Metab 1990; **10**: 358–64.

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丁基苯酞对大鼠局灶性脑缺血和重灌后脑内 TXB_2 和 6-keto-$PGF_{1\alpha}$ 含量的影响[1]

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关键词 丁基苯酞；血栓素 B_2；6-酮前列腺素 $F_{1\alpha}$；阿司匹林；局灶性脑缺血

目的：观察丁基苯酞(NBP)对大鼠局灶性脑缺血及重灌后海马，纹状体和皮层中 TXB_2 及 6-keto-$PGF_{1\alpha}$ 含量的影响. **方法**：尼龙线栓塞法造成大鼠局灶性脑缺血模型. TXB_2 和 6-keto-$PGF_{1\alpha}$ 用放免法测定.

结果：NBP 10 mg·kg^{-1}治疗对缺血重灌注后脑组织中 TXB_2 的产生具有抑制作用，但对 6-keto-$PGF_{1\alpha}$的产生无明显作用．NBP 20 mg·kg^{-1}治疗后、重灌 5 min 缺血脑组织中 TXB_2 和重灌后 30 min 时 6-keto-$PGF_{1\alpha}$含量皆明显减少．NBP 20 或 10 mg·kg^{-1}皆明显提高 PGI_2/TXA_2 的比值．而阿司匹林(20 mg·kg^{-1})除重灌 5 min 提高纹状体 PGI_2/TXA_2 的比值外，在其它时间点上均无提高作用．结论：NBP 提高缺血脑组织中 PGI_2/TXA_2 的比值，可能有利于改善缺血脑组织的微循环状态．

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Physostigmine blocked nicotinic acetylcholine receptors in rat sympathetic ganglion neurons[1]

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KEY WORDS sympathetic ganglia; nicotinic receptors; physostigmine; allosteric regulation; patch-clamp techniques

AIM: To study the blocking mechanism of physostigmine (Phy) on nicotinic acetylcholine receptors (NAChR) in sympathetic neurons. **METHODS**: The whole-cell patch-clamp technique was used to observe the effects of Phy on NAChR in the cultured sympathetic neurons from neonatal rat superior cervical ganglia (SCG). **RESULTS**: Phy 5 - 20 μmol·L^{-1} inhibited neuronal NAChR in a concentration-dependent manner and accelerated the desensitization of NAChR. Changing the membrane potential from - 50 to - 90 mV did not affect the blocking effect of Phy. Phy 200 μmol·L^{-1} did not induce any noticeable response in SCG neurons. **CONCLUSION**: Phy blocked NAChR in the sympathetic ganglion neurons by interacting with the allosteric sites out of the binding sites and the open ionic channels of the receptors. Phy did not possess excitative effect on NAChR in SCG neurons.

Physostigmine (Phy) is not only a reversible cholinesterase inhibitor, but also exhibits postsynaptic effects resulting primarily from a direct interaction with nicotinic acetylcholine receptors (NAChR)[1-5]. On muscle[1-3], electric organ[4], and mouse tumor cells[5], the inhibitory effect of Phy on NAChR resulted from blockade of the ionic channels in an open conformation. Apart from the depression, Phy also displayed a direct excited effect on muscular[2,3] and electrical organ NAChR channels even though NAChR were in the desensitization state[4].

Few experiments have been conducted about the blocking effect of Phy on neuronal NAChR. It was only reported that Phy could reduce the depolarized response of rabbit superior cervical ganglia (SCG) neurons to ACh[9]. As the structure and biological characteristics of neuronal NAChR are different from those of muscle NAChR[6], Phy may present diverse pharmacological properties on neuronal NAChR. In this experiment, we used the cultured rat SCG neurons to elucidate the blocking mechanism and analyze the reaction site of Phy on neuronal NAChR.

MATERIALS AND METHODS

Cell culture SCG were isolated from neonatal Wistar rats (1 d). The dissociating method was described in our previous work[7]. Briefly, the ganglia were cut into small pieces and digested with 0.25 % trypsin. The suspension was spun at 500 × *g* for 2 min. The pellet was resuspended in DMEM containing 10 % horse serum. The dissociated neurons were transferred to 35-mm tissue culture dishes and were cultured at 37 ℃ in 95 % O_2 - 5 % CO_2 for 7 - 9 d. In this period,

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