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Effects of Sodium Nitroprusside on the Catecholamine Synthetic Pathway in the Adrenal Medulla of Rats

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ABSTRACT—We studied the effect of sodium nitroprusside (SNP), a nitric oxide (NO) donor, on tyrosine hydroxylase (TH) activity and epinephrine and norepinephrine levels in the adrenal medulla of rats. TH activity and the levels of epinephrine and norepinephrine in the adrenal medulla of the SNP+nicotine (Nic)-treated group were increased significantly compared to those in the control, Nic-treated and SNP-treated groups. Furthermore, methylene blue inhibited this increase in TH activity. The data suggest that the NO derived from SNP may increase TH through the guanylyl cyclase pathway in the presence of Nic.

Keywords: Nitric oxide, Sodium nitroprusside, Tyrosine hydroxylase, Catecholamine, Adrenal medulla

Nitric oxide (NO) is recognized as the endothelium-derived relaxing factor responsible for vascular dilation (1, 2); it also exerts an effect on the nervous system (3, 4). Large quantities of type I NO synthase (NOS) and NO are known to be present in the brain and neural tissue. In relation to catecholamine release, NO has been implicated in a few studies as both a potentiator and an inhibitor. Yamamoto et al. reported that NO potentiates norepinephrine release in the rat mesenteric artery (5). In contrast, Macarthur et al. reported that NO inhibits norepinephrine release by high K^+ stimulation in the PC12 cell (6). However, the effect of NO on the catecholamine synthetic pathway is unknown. To evaluate the effect of NO on tyrosine hydroxylase (TH), which is a rate-limiting enzyme of catecholamine synthesis, we studied the effect of sodium nitroprusside (SNP), an NO donor, on TH activity, as well as epinephrine and norepinephrine levels in the adrenal medulla of rats.

MATERIALS AND METHODS

Animals

All studies were performed according to the "Guiding Principles for the Care and Use of Laboratory Animals" of The Japanese Pharmacological Society. Fifteen-week-old male Wistar rats were housed in a semi-barrier system in a room with controlled temperature (23±1℃), humidity (55±5%) and lighting (light from 6 AM to 6 PM).

Incubation study

Tissue preparation was performed as previously described (7). The tissue was prewarmed at 37℃ and incubated in 1 ml of Krebs' buffer for 90 min. The buffer composition was as follows: 118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 11 mM glucose, 0.57 mM ascorbic acid, 0.03 mM EDTA.2Na, with superoxide dismutase (30 IU/ml), pH 7.4, in the presence or absence of nicotine (Nic, 10^{-5} M). In the dose-response experiment, SNP was added to the incubation medium at 10^{-7}–10^{-3} M, and Nic was added to the incubation medium at 10^{-7}–10^{-4} M in the presence of SNP (10^{-4} M). In the other experiments, 10^{-4} M SNP added. Methylene blue (MB) was used at 10^{-4} M. Hexamethonium was used at 10^{-5} M.

Epinephrine and norepinephrine analysis

The method of epinephrine and norepinephrine analysis was described previously (8). In brief, the tissues were homogenized in a glass tissue grinder with 0.05 M perchloric acid. The epinephrine and norepinephrine were extracted with aluminum oxide and measured by HPLC (510 pump; Waters, Milford, MA, USA) with an electrochemical detector (460 detector, Waters) and a column (Cosmosil 5C18-AR packed column, 4.6×150 mm; Nacalai Tesque Co., Kyoto). The mobile phase consisted of the following components: 50 mM sodium acetate, 20 mM citric acid, 3.75 mM sodium octyl sulfate, 1 mM di-*n*-butylamine, 0.134 mM EDTA and 5% (V/V)

methanol. All separations were performed isocratically at a flow-rate of 0.9 ml/min at 35℃. The detector potential was maintained at +0.65 V.

TH activity analysis

TH activity was measured using a modified version of the method of Nagatsu et al. (9), which was described previously (8). In brief, the tissues were homogenized with 0.25 M sucrose (50 vol.) in a glass tissue grinder. The homogenate was incubated with 1 mM L-tyrosine and 1 mM 6-methyl-5,6,7,8-tetrahydropterine at 37℃ for 10 min. DOPA was extracted with aluminum oxide and Amberlite CG-50 (Aldrich Chem. Co., Milwaukee, WI, USA) and measured by HPLC (510 pump, Waters) with an electrochemical detector (460 detector, Waters) and a column (Cosmosil 5C18-AR packed column, 4.6×150 mm; Nacalai Tesque Co.). The mobile phase consisted of the following components: 50 mM sodium acetate, 20 mM citric acid, 12.5 mM sodium octyl sulfate, 1 mM di-*n*-butylamine and 0.134 mM EDTA. All separations were performed isocratically at a flow-rate of 0.6 ml/min at 28℃. The detector potential was maintained at +0.65 V. TH activity was calculated as the amount of DOPA formed from tyrosine per gram of tissue per hour.

NO_2/NO_3 (NOX) level analysis

One hundred microliters of incubation medium was mixed with 400 μl of distilled water and 300 μl of 3 M NaOH. After 5 min, 300 μl of 5% $ZnSO_4$ was added, and the mixture was centrifuged at 12000×*g* for 20 min at 4℃. The supernatant was passed through a 0.45-μm filter, and an aliquot (100 μl) was used for NOX analysis. NOX level was determined with an autoanalyzer (TCI-NOX1000; Tokyo Kasei Kogyo Co., Tokyo). Samples were reduced on a copper-plated cadmium column in which NO_3^- was reduced to NO_2^-, which reacts with Griess reagent (1% sulfonamide, 0.1% *N*-naphthylethyl-ethylenediamine dihydrochloride, and 5% HCl) to form a purple azo dye, and absorbance was detected at 540 nm.

Statistical analyses

Data are presented as mean±S.E.M. The statistical difference between mean values was analyzed by ANOVA and Dunnett's multiple range test. A probability value less than 0.05 was considered statistically significant.

RESULTS

The time course of TH activity in the adrenal medulla incubated with SNP and/or Nic is shown in Fig. 1. TH activity increased time dependently (0−30 min, from 222.0±18.9 to 409.7±24.8 μg/g tissue/hr) and reached equilibrium after 30 to 90 min (90 min, 414.0±14.2 μg/g tissue/hr) during the incubation.

SNP treatment in the presence of Nic increased TH activity in the adrenal medulla in a dose-related (10^{-7} to 10^{-3} M) manner (from 271.8±76.1 to 664.1±106.2 μg/g tissue/hr, Fig. 2).

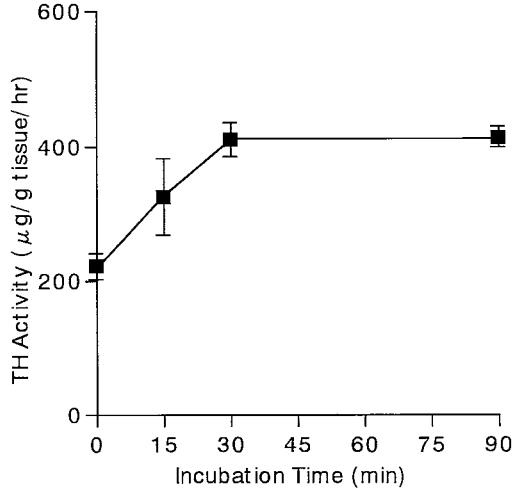


Fig. 1. The time course of tyrosine hydroxylase (TH) activity in the adrenal medulla incubated with nicotine and sodium nitroprusside. Values are each a mean±S.E.M. Nicotine (10^{-5} M) and sodium nitroprusside (10^{-4} M) added to the incubation medium. Three rats from each of the groups were used.

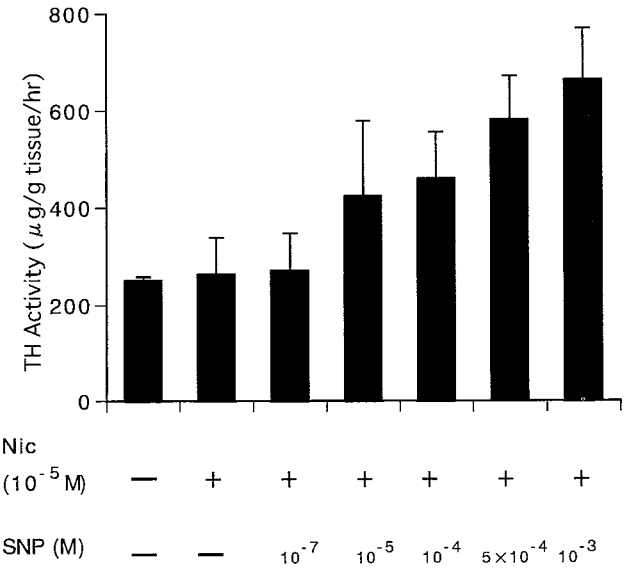


Fig. 2. Bar graphs show effects of nicotine (Nic) and sodium nitroprusside (SNP) on tyrosine hydroxylase (TH) activity in the adrenal medulla of the rat. Values are each a mean±S.E.M. − indicates no drug was added to the incubation medium; + indicates Nic (10^{-5} M) was added to the incubation medium. SNP was added to the incubation medium at 10^{-7}−10^{-3} M. Three rats from each of the groups were used.

Treatment with 10^{-3} M SNP without Nic significantly increased TH activity (control: 208.8±5.3, SNP 10^{-3} M: 335.4±32.9 μg/g tissue/hr; Fig. 3).

Nic treatment in the presence of SNP (10^{-4} M) increased TH activity in the adrenal medulla in a dose-related (10^{-7} to 10^{-4} M) manner (from 210.5±6.0 to

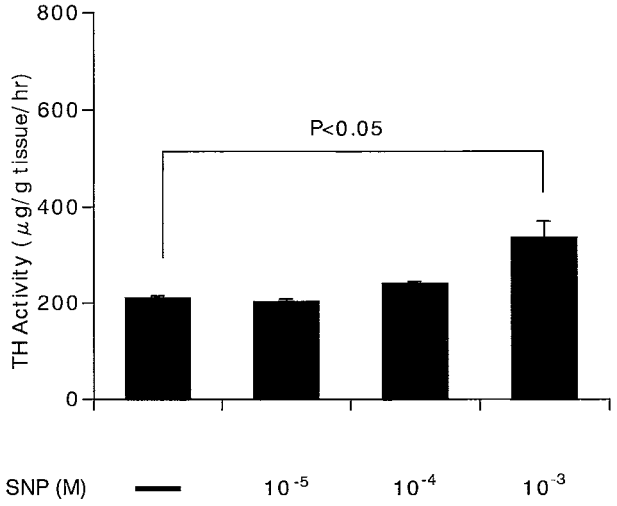


Fig. 3. Bar graphs show the effect of sodium nitroprusside (SNP) on tyrosine hydroxylase (TH) activity in the adrenal medulla of the rat. Values are each a mean±S.E.M. − indicates no drug was added to the incubation medium. SNP was added to the incubation medium at 10^{-5}–10^{-3} M. Three rats from each of the groups were used.

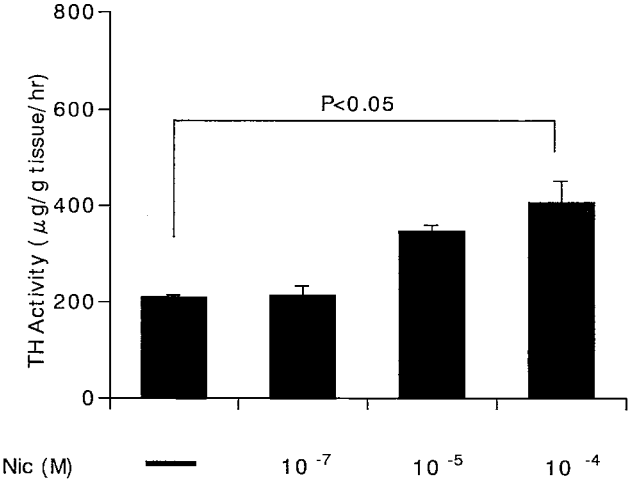


Fig. 4. Bar graphs show effects of nicotine (Nic) and sodium nitroprusside (SNP) on tyrosine hydroxylase (TH) activity in the adrenal medulla of the rat. Values are each a mean±S.E.M. − indicates Nic was not added to the incubation medium; Nic was added to the incubation medium at 10^{-7}–10^{-4} M. SNP (10^{-4} M) was added to the incubation medium. Three rats from each of the groups were used.

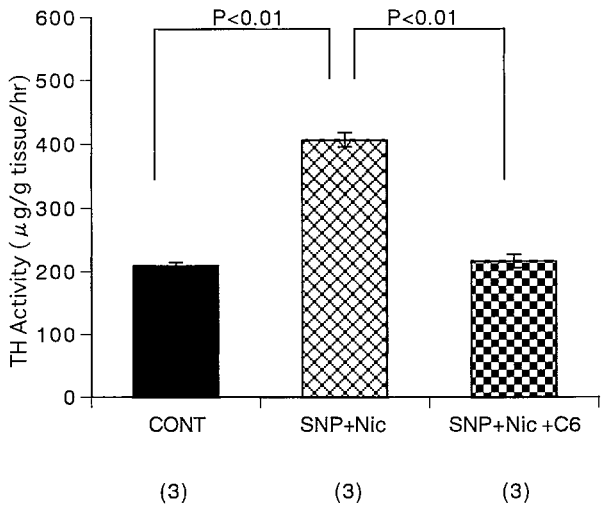


Fig. 5. Bar graphs show effects of hexamethonium (C6) on increases of nicotine (Nic) and sodium nitroprusside (SNP)-treated tyrosine hydroxylase (TH) activity in the adrenal medulla of the rat. Values are each a mean±S.E.M. CONT indicates control rats; SNP+Nic indicates SNP (10^{-4} M) and Nic (10^{-5} M) added to the incubation medium; SNP+Nic+C6 indicates SNP (10^{-4} M), Nic (10^{-5} M) and C6 (10^{-5} M) added to the incubation medium. Number of rats is shown in parentheses.

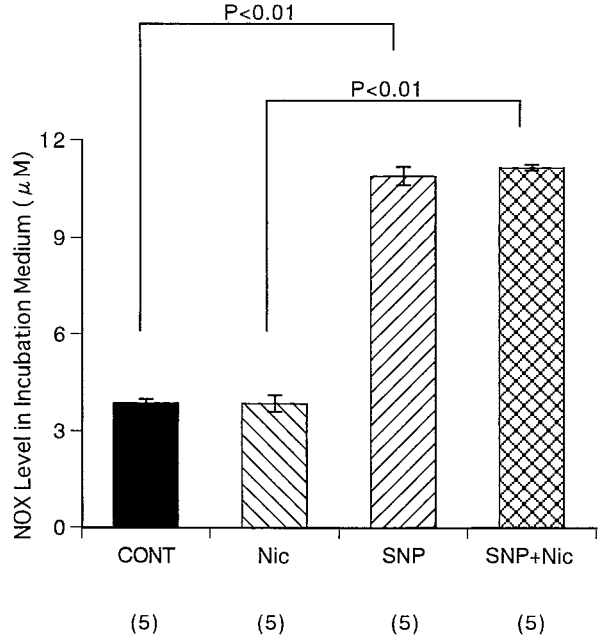


Fig. 6. Bar graphs show effects of nicotine (Nic) and sodium nitroprusside (SNP) on NO_2/NO_3 (NOX) levels in the incubation medium. Values are each a mean±S.E.M. CONT indicates control rats; Nic indicates Nic (10^{-5} M) was added to the incubation medium; SNP indicates SNP (10^{-4} M) was added to the incubation medium; SNP+Nic indicates SNP (10^{-4} M) and Nic (10^{-5} M) were added to the incubation medium. Number of rats is shown in parentheses.

406.7±43.4 μg/g tissue/hr, Fig. 4).

Hexamethonium inhibited the increases in TH activity associated with SNP and Nic treatment (216.4±10.4 μg/g tissue/hr, Fig. 5).

The NOX levels in incubation medium of the various groups are shown in Fig. 6. NOX levels in the SNP (10.9±0.29 μM) and SNP+Nic (11.16±0.09 μM) groups were significantly higher than in the control (3.85±0.15 μM) and Nic (3.85±0.26 μM) groups.

The effects of SNP and Nic on epinephrine and norepinephrine levels in the adrenal medulla are shown in Fig. 7. Both the epinephrine (1983.9±179.3 μg/g tissue) and norepinephrine (164.4±12.6 μg/g tissue) levels in the SNP+Nic group were significantly higher than those in the control (epinephrine: 1457.7±54.6, norepinephrine: 110.9±5.3 μg/g tissue), Nic (epinephrine: 1607.8±153.9, norepinephrine: 124.8±17.1 μg/g tissue), or SNP (epinephrine: 1458.6±199.5, norepinephrine: 81.9±9.4 μg/g tissue) groups.

TH activity in the SNP+Nic group (319.4±57.0 μg/g tissue/hr) was significantly higher than that in the control (168.9±52.9 μg/g tissue/hr) group (Fig. 8). TH activities in the Nic (245.6±63.1 μg/g tissue/hr) and SNP (249.7±72.3 μg/g tissue/hr) groups were also higher than in the control group, but the difference was not significant.

When MB was added to the incubation mediun of the SNP+Nic group, TH activity in the resulting SNP+MB+Nic group (240.0±32.0 μg/g tissue/hr) was significantly lower than in the group with SNP+Nic (472.0±64.0 μg/g tissue/hr) alone (Fig. 9). TH activity in the SNP+MB+Nic group recovered to the level of the control group (216.0±80.0 μg/g tissue/hr). Furthermore, TH activity did not differ significantly between the MB group (216.0±32.0 μg/g tissue/hr) and the control group.

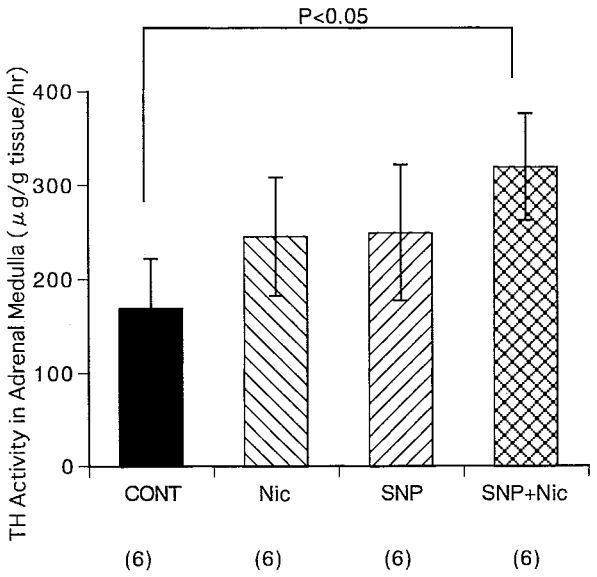
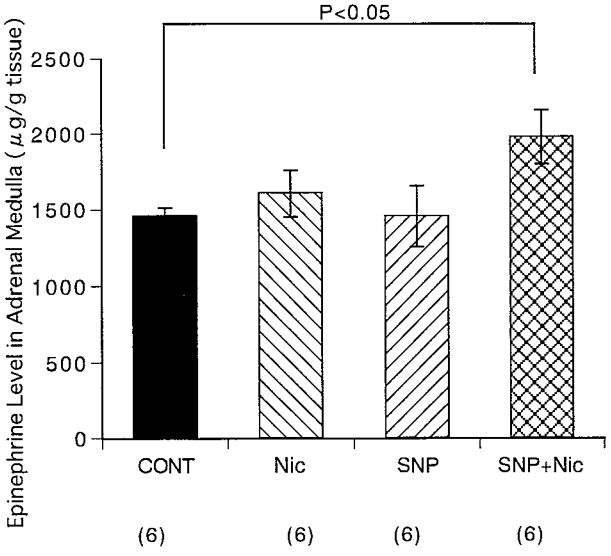


Fig. 8. Bar graphs show effects of nicotine and sodium nitroprusside on tyrosine hydroxylase (TH) activity in the adrenal medulla of rats. Values are each a mean±S.E.M. Definitions are as in the Fig. 6 legend. Number of rats is shown in parentheses.



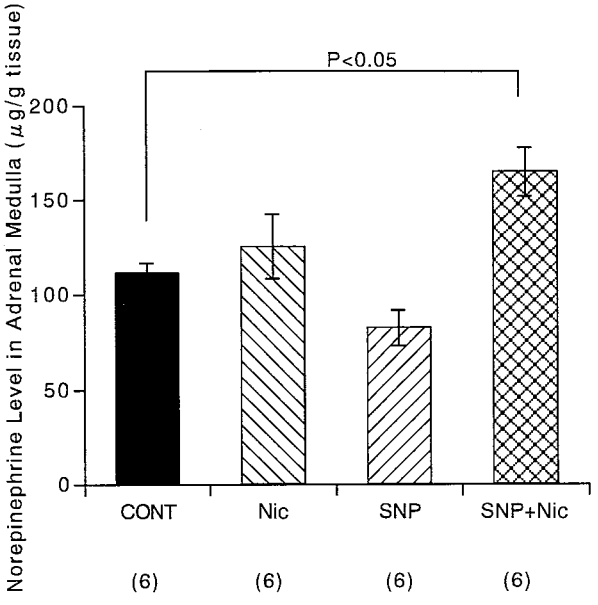


Fig. 7. Bar graphs show effects of nicotine and sodium nitroprusside on epinephrine and norepinephrine levels in the adrenal medulla of the rat. Values are each a mean±S.E.M. Definitions are as in the Fig. 6 legend. Number of rats is shown in parentheses.

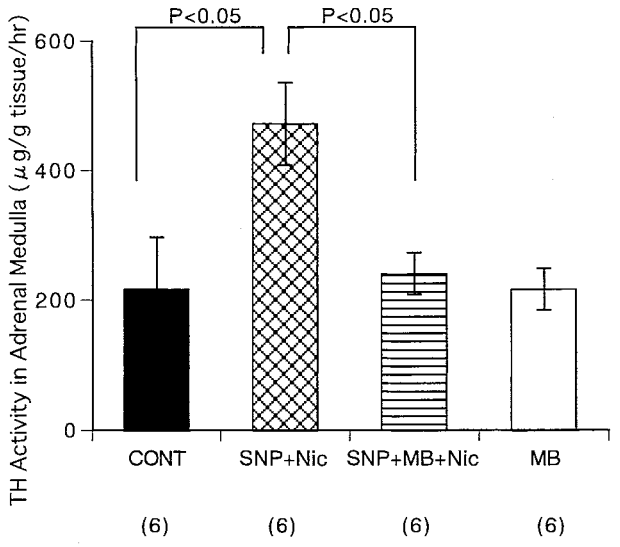


Fig. 9. Bar graphs show effects of methylene blue (MB) on tyrosine hydroxylase (TH) activity in the nicotine (Nic) and sodium nitroprusside (SNP)-treated adrenal medulla of the rat. Values are each a mean±S.E.M. CONT indicates control rats; SNP+Nic indicates SNP (10^{-4} M) and Nic (10^{-5} M) were added to the incubation medium; SNP+MB+Nic indicates SNP (10^{-4} M), MB (10^{-4} M) and Nic (10^{-5} M) were added to the incubation medium; MB indicates MB (10^{-4} M) was added to the incubation medium. Number of rats is shown in parentheses.

DISCUSSION

The major findings of the present study are that SNP treatment in the presence of Nic increases TH activity as well as epinephrine and norepinephrine levels in the adrenal medulla. In addition, MB inhibits this increase in TH activity.

SNP induces a biological response through the production of NO (10). Both epinephrine and norepinephrine levels in the adrenal medulla of the SNP+Nic-treated group were significantly higher than those in the control, SNP and Nic groups. Since TH is a rate-limiting enzyme of epinephrine and norepinephrine synthesis, these changes in epinephrine and norepinephrine levels may reflect the effect of SNP and Nic on TH activity. Our data suggest that the increased NO derived from SNP affected the catecholamine synthetic pathway in the adrenal medulla. We found that MB, which is a guanylyl cyclase inhibitor, abolished the SNP-induced enhancement of TH activity. SNP and NO have been shown to stimulate guanylyl cyclase and increase neuronal levels of cyclic GMP (11, 12). NO derived from SNP may increase TH through the guanylyl cyclase pathway.

In the present study, we showed that SNP increased NOX levels in both the SNP and Nic+SNP-treated groups. However, TH activity in the adrenal medulla was increased only in the Nic+SNP-treated group. Furthermore, hexamethonium abolished this TH activity increase by Nic+SNP treatment. It is possible that the effect of SNP in increasing TH activity requires nicotinic stimulation. There are two possible explanations for this.

First, the effect of SNP on TH activity may be related to TH phosphorylation and/or TH gene expression by protein kinase. The phosphorylation and/or gene expression of TH is a primary cellular mechanism for controlling the activity of this enzyme (13). Roskoski and Roskoski reported that cyclic GMP activates TH activity (14). They suggested that the activating mechanism may involve protein kinase A and/or protein kinase G (15). It is well known that protein kinase A is involved in the phosphorylation and activation of TH (16). In addition, Hiremagalur et al. reported that Nic increases expression of the TH gene through the protein kinase A pathway (17). NO increase of cyclic GMP has been established. Therefore, cyclic GMP increased by NO derived from SNP may have a synergistic action on the TH activating mechanism by nicotinic stimulation through the protein kinase A system.

Second, the effect of SNP on TH activity may be related to the intracellular Ca^{2+} increasing mechanism. When the intracellular Ca^{2+} level is increased, TH is activated. Recently, Oset-Gasque et al. reported that NO donors increased intracellular Ca^{2+} levels in the bovine chromaffin cell (18). Indeed, our present study demonstrated that high dose (10^{-3} M) of SNP (without Nic) increased TH activity slightly. Marley et al. demonstrated that the effect of Nic on TH activation required extracellular Ca^{2+} in cultured bovine chromaffin cells (19). It is possible that NO derived from SNP may increase intracellular Ca^{2+} synergistically with Nic.

In the present study, we used SNP, an NO donor, to evaluate the role of NO in the catecholamine synthetic pathway. We confirmed that NOX, an NO metabolite, in the incubation medium was increased by SNP treatment. Therefore, our data suggest that exogenous NO derived from SNP caused the TH activation and increases in epinephrine and norepinephrine levels in the adrenal medulla. Recently, histochemical studies have indicated that type I NOS and TH are colocalized in the nervous system (20, 21). These reports suggest that many catecholaminergic nerves as well as non-catecholaminergic nerves have the capacity to synthesize NO and that NO may play a role in some physiologic autonomic controls (21). These data indicate that NO produced endogenously by type I NOS in the adrenal medulla may play a role in catecholamine synthesis.

In conclusion, NO-derived SNP in the presence of Nic may be related to the increase in TH activity through the guanylyl cyclase pathway.

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