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EFFECTS OF NITROPRUSSIDE AND NITROGLYCERIN ON cGMP CONTENT AND PGI_2 FORMATION IN AORTA AND VENA CAVA

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Abstract—Nitroprusside (NP) and nitroglycerin (NG) are potent vasodilators that are used clinically on the basis of their abilities to cause relaxation of smooth muscle. *In vitro*, both agents cause activation of guanylate cyclase, resulting in increased intracellular cGMP. They also have effects on arachidonate metabolism. Despite apparent similarities in their mechanisms of action, the two drugs have different therapeutic applications based in part on differences in their effectiveness on the arterial and venous systems *in vivo*. To understand better their target tissue preference, slices of aorta and vena cava were incubated with the agents; cGMP and the vasodilatory prostanoid, prostacyclin, were quantified. NP was more effective in increasing the cGMP content of aorta than of vena cava; it was more active than NG in both tissues. Prostaglandin formation by vascular tissue was influenced by the preliminary equilibration period. Under optimal conditions, it appeared that NG enhanced prostacyclin formation in aorta more than did NP. This *in vitro* model for NP and NG action may be useful in studying the mechanisms of action of these and other vasoactive agents.

Nitroprusside (NP), nitroglycerin (NG) and related compounds are used clinically in the treatment of a number of diseases of the cardiovascular system, such as heart failure, hypertension, and angina [1–4]. These agents are known to be potent vasodilators [1–4]; *in vitro*, the drugs cause a rapid relaxation of smooth muscle [5–8]. Although the biochemical basis for the action of these drugs has not been defined completely, it is known that these compounds can activate guanylate cyclase and thereby increase cGMP content [5–7, 9]. Using partially purified preparations of guanylate cyclase, it has been shown that nitric oxide (NO) and compounds from which NO can arise activate the enzyme in the presence of heme [9–15]. Activation of guanylate cyclase leads to increased tissue cGMP content. Since incubation of smooth muscle with cGMP analogs results in relaxation [8, 16–18], cGMP-dependent events have been implicated in the action of NP and NG on smooth muscle. Prostacyclin also causes relaxation of arterial smooth muscle [19–21]. NG was found to induce prostacyclin formation by human veins, coronary arteries, and human endothelial cells derived from umbilical veins [22–24]. Thus, the effect of NG may be mediated by two pathways, one involving cGMP and the other, prostacyclin.

Although NG and NP appear to have similar mechanisms of action, it is apparent that the drugs exert their effects on different target tissues and, consequently, differ in therapeutic effectiveness [25]. NP exhibits balanced action on both the venous and arterial systems [25–29]. In contrast, NG appears to have more venodilator than arterial dilating action [26, 29]. The effects of NG on venous circulation may be somewhat greater than those of NP [25, 28]. These studies are consistent with the hypothesis that tissues possess different mechanisms for the processing of the nitro compounds, and/or that the drugs exert differential effects on other biochemical pathways. Since both drugs can activate guanylate cyclase and increase tissue cGMP content, the effects of NP and NG on rat aorta and vena cava cGMP content were examined. In addition, since smooth muscle relaxation may be secondary to the local production of vasodilating agents, the effects of both drugs on prostacyclin formation were examined. The results of these studies support a differential effect of NG and NP on aorta and vena cava.

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METHODS

Preparation of tissues and determination of cyclic GMP. Male, Osborne Mendel rats (NIH strain, 150–200 g) were decapitated; thoracic aorta and vena cava were rapidly removed to Krebs–Ringer Tris-buffer, pH 7.4, containing bovine serum albumin (3 mg/ml) and glucose (1 mg/ml) (buffer A) at 0–4° [30]. Vessels were opened longitudinally and aorta was cut into 3 mm segments. Samples of aorta (six to nine pieces, 70–100 mg wet weight) were then incubated at 37° in 3 ml of buffer A which was changed at 30, 60, and 90 min unless otherwise noted. Samples of vena cava (20–30 mg) were incubated in 1.5 ml of buffer A which was replaced after 30 min. NP (Sigma, St. Louis, MO) or NG was dissolved in ice-cold water immediately before use. Ten minutes after the last buffer change, 20 μl of drug solution or

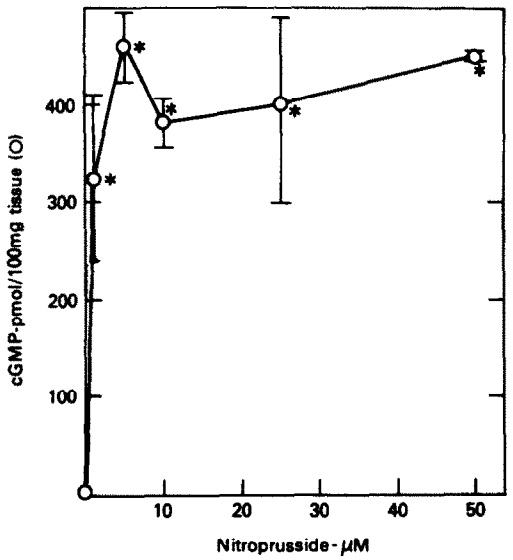

Fig. 1. Effect of NP concentration on cGMP content of aorta. Samples of aorta were incubated with the indicated concentration of NP for 60 sec at 37° before determination of cGMP content. Key: (*) NP vs basal, $P < 0.05$.

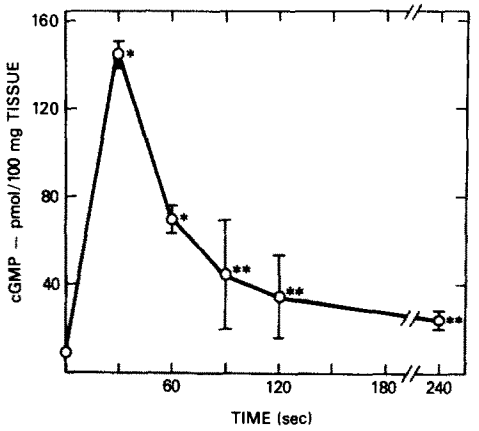

Fig. 3. Effect of NG on the time course of aorta cGMP accumulation. Aorta was incubated at 37° with 3.5 μM NG for the time indicated. Key: (*) NG vs basal, $P < 0.005$ and (**) P, insignificant.

cold water were added to tissues. After the indicated time at 37°, samples of medium were removed for prostacyclin assay and ice-cold 15% trichloroacetic acid (final concentration 5%) was added to the tissue. Samples at 0 time were taken as quickly as possible (~10 sec) after addition of NP or NG. Samples were frozen quickly in an ethanol–dry ice bath, subsequently thawed at 0° and homogenized with Polytron using three 20-sec bursts. After centrifugation, trichloracetic acid was extracted from the supernatant fraction with Freon octylamine [31] and samples were taken for radioimmunoassay of cGMP (New England Nucler Kit, New England Nuclear, Wilmington, DE). Data reported are the average of values or the actual values from duplicate incubations; bars in Figs. 1–7 indicate the range unless it was within the symbol.

Prostacyclin assays. Samples (200 μl) of medium were taken just before, and at the indicated time after, addition of NG for radioimmunoassay (New England Nuclear) of prostacyclin as its hydrolysis product 6-keto-$PGF_{1\alpha}$. Prostacyclin production, reported as Δ 6-keto-$PGF_{1\alpha}$, is the difference between values for samples taken before, and at the indicated time after, addition of agonist. The average of values or the actual values from duplicate incubations are recorded.

RESULTS

NP caused a dose- and time-dependent increase in the cGMP content of aorta (Figs. 1 and 2). Maximal effects on cGMP accumulation were observed with 5 μM NP (Fig. 1). Following addition of 50 μM NP, cGMP content rose rapidly, reaching a maximum of >100 times basal at about 30–60 sec and declining

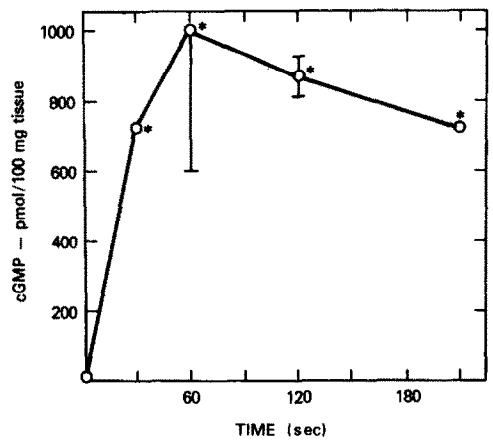

Fig. 2. Effect of NP on the time course of aorta cGMP accumulation. Samples of aorta were incubated with 50 μM NP at 37° for the indicated time. Key: (*) NP vs basal, $P < 0.05$.

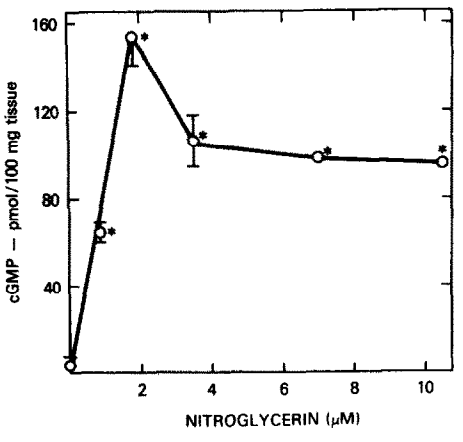

Fig. 4. Effect of NG concentration on cGMP content of aorta. Samples of aorta were incubated with the indicated concentration of NG for 30 sec at 37°. Key: (*) NG vs basal, $P < 0.01$.

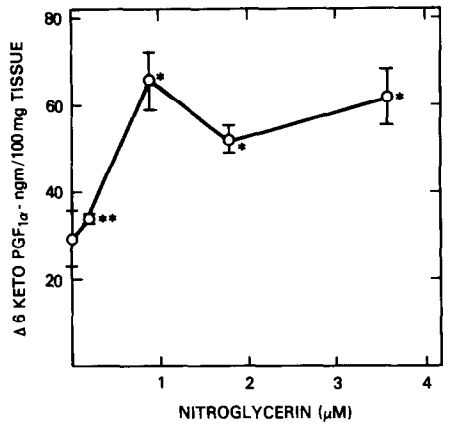

Fig. 5. Effect of NG on 6-keto-$PGF_{1\alpha}$ production by aorta. Samples were derived from the same experiment as in Fig. 4. Samples of medium were taken just before, and 30 sec after, addition of NG for the determination of 6-keto-$PGF_{1\alpha}$. Key: (*) NG vs basal, $P < 0.025$, and (**) P, insignificant.

thereafter, although it was still elevated significantly at 3.5 min (Fig. 2). NP (2–50 μM) during a 60-sec incubation had no effect on prostaglandin I_2 (PGI_2) production (data not shown).

NG also increased cGMP content of the aorta (Figs. 3 and 4). The response of the aorta to 3.5 μM NG was rapid and transient, reaching a peak in ~30 sec. cGMP content declined to basal levels in 2 min (Fig. 3). Maximal effects were produced with ~2 μM (Fig. 4) and were much less than those with NP.

NG enhanced 6-keto-$PGF_{1\alpha}$ production with maximal formation observed at concentrations from 0.9 to 3.5 μM (Fig. 5); with 7 μM or greater, 6-keto-$PGF_{1\alpha}$ formation was less (data not shown). Indomethacin blocked the NG-dependent increase in 6-keto-$PGF_{1\alpha}$ but did not inhibit the increase in cGMP content of aorta caused by NG or NP (Table 1).

The sensitivity of the aorta to NG and, to a lesser extent, NP appeared to be dependent on the equilibration of the tissue in medium prior to addition of drug. As noted in Table 2, aorta that was equilibrated in medium for only 30 min showed a 6-fold response

Table 2. Effects of NG or NP on formation of cGMP in aorta

Addition	cGMP content (pmol/100 mg tissue)	
	30 min	90 min
None	0.7, 0.9	1.2, 0.9
NG, 3.5 μM	6.0, 4.7	25, 70
NP, 25 μM	142, 155	174, 191

Segments of aorta were equilibrated for 30 min without change of medium or for 90 min with three changes of medium before incubation for 45 sec at 37° with additions as indicated.

to NG; this effect of NG was magnified roughly 9-fold by increasing the time of incubation of aorta prior to exposure to drug from 30 min to 90 min with three changes of medium. Increasing the equilibration time of tissue from 30 min to 90 min only altered the NP effect slightly (Table 2).

NP and NG increased cGMP content of rat vena cava in a time-dependent manner (Figs. 6 and 7). Maximal effects of NP were observed at 50 μM, those of NG at 7 μM. The levels of cGMP produced following addition of NP were 6-fold higher than those observed in the presence of NG. In contrast to the aorta where maximal cGMP levels were achieved within 60 sec following addition of agent, in the vena cava maximal increases in cGMP content were produced with either NP or NG after 180 sec of incubation (Figs. 6 and 7). The duration of equilibration of the vena cava was not as critical to NG or NP responsiveness as was the case in the aorta (Table 3). Since incubation of vena cava with multiple medium changes as done with aorta led to a loss of NG effects on cGMP (Table 3), the standard incubation was 30 min with no change of medium. Under these conditions, formation of 6-keto-$PGF_{1\alpha}$ by vena cava was ~28 ng/100 mg. Under these and a variety of similar conditions, no significant effect of NP or NG on 6-keto-$PGF_{1\alpha}$ could be observed (data not shown).

DISCUSSION

In the studies reported here, NP was clearly more effective in increasing cGMP content of aorta than

Table 1. Effect of indomethacin on 6-keto-$PGF_{1\alpha}$ formation by aorta

	cGMP (pmol/100 mg tissue)		Δ6-keto-$PGF_{1\alpha}$ (ng/100 mg tissue)	
	Addition		Addition	
	None	Indo	None	Indo
Basal	10, 11, 12	12, 13, 14	40, 30	22, 22
NG	98, 33	164, 112	64, 40	13, 19
NP	323, 768	470, 574	28, 31	27, 13

Samples of medium (following a 1.5 hr incubation and three medium changes) were taken just before, and 30 or 60 sec after, addition of NG (3.5 μM) or NP (10 μM), respectively, with or without 1 μM indomethacin (Indo) (Sigma) for the determination of 6-keto-$PGF_{1\alpha}$.

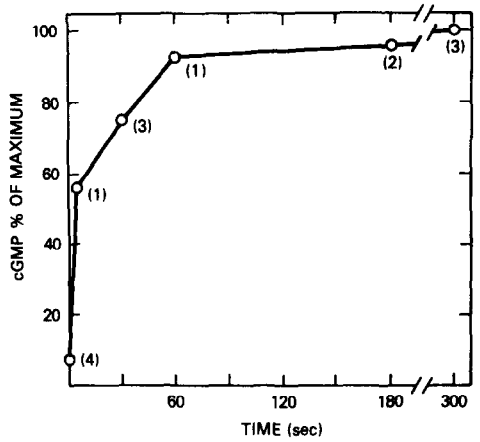

Fig. 6. Effect of NP on cGMP content of vena cava. Samples of vena cava were incubated for the indicated time with 50 μM NP. cGMP content was expressed as a percentage of the maximum in each experiment. The actual value or the mean of the values from the number of experiments shown in parentheses are plotted. Zero time cGMP content ranged from 2.7 to 5 pmol/100 mg tissues. One hundred percent maximum: cGMP content ranged from 25 to 75 pmol/100 mg tissue.

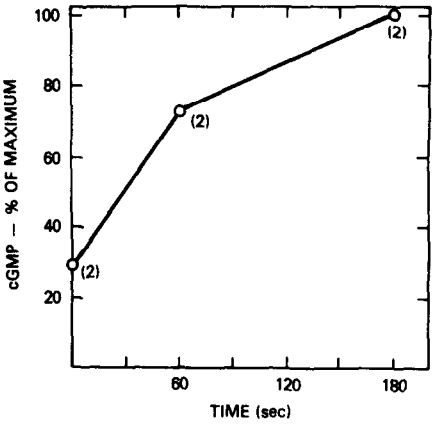

Fig. 7. Effect of NG on cGMP content of vena cava. Samples of vena cava were incubated with 7 μM NG for the indicated time. Data are presented as in Fig. 6. At zero time, cGMP content ranged from 1.5 to 1.7 pmol/100 mg tissue. At 180 sec it was 5.2 to 6 pmol/100 mg tissue.

of vena cava; it was also more potent than NG in elevating the cGMP content of both aorta and vena cava. NG, on the other hand, was more effective than NP in increasing prostacyclin formation in aorta; NG-dependent prostacyclin formation was blocked by indomethacin. In the absence of an equilibration period, NG had no effect on prostanoid formation; increasing the time of incubation of aorta prior to addition of the drugs markedly enhanced effects of NG on cGMP accumulation and prostanoid formation. In contrast, with vena cava, the effects of both NP and NG on cGMP content appeared to be independent of time of incubation before drug addition.

The therapeutic effects of both NP and NG appear to result from their vasodilatory properties [1–4]. Physiologically, both the arterial and venous circulation respond to NP and NG [4]; NP is more effective than NG on the arterial bed, whereas they appear to be equally effective on the venous circulation. Some of the vasodilatory properties of these drugs may result from their effects on cGMP content [5–7, 9–15] and prostanoid formation [22–24, 32]. In this light, it is interesting that NP was more potent than NG in enhancing cGMP especially in aorta, whereas NG was more active in increasing prostacyclin release. cGMP elevation in aorta in the presence of NP was sustained for a longer time than that obtained with NG; it is not clear whether this transient elevation in cGMP is related to the development of tolerance to these agents *in vivo*. The relative effectiveness *in vivo* of the two agents may depend, therefore, on the importance of cGMP and arachidonate metabolites, among other factors, in promoting vasodilatation in different vascular beds. Since, however, the vascular components of the arterial system differ in responsiveness to these agents [25, 33], the relative effectiveness of NG and NP on cGMP and prostanoid formation in aorta and vena cava may not reflect that of the entire arterial and venous circulation.

Table 3. Effect of NG or NP on cGMP content of vena cava and aorta after equilibration for 30 or 90 min

Tissue		cGMP content (pmol/100 mg tissue) Basal	NG	NP
Aorta	30 min	4 ± 2 (42)	21 ± 3* (4)	251 ± 7.9‡, ‖ (4)
	90 min	4 ± 2 (42)	81 ± 4.5* (10)	290 ± 15.7‡, ‖ (4)
Vena cava	30 min	1.8 ± 0.7 (13)	4.2 ± 1.1† (10)	62 ± 5.3§, ‖ (6)
	90 min	3.4 ± 1.4 (8)	2.6 ± 1.5 (16)	64 ± 2.9‡, ‖ (3)

Tissues were equilibrated for either 30 min without change of medium or for 90 min with three changes before incubation with 7 μM NG or 25 μM NP for 30 sec (aorta) or 3 min (vena cava). Data represent the mean ± SEM; numbers in parentheses represent the number of samples.

*, † NG vs basal: * $P < 0.001$, and † $P < 0.05$.

‡, § NP vs basal: ‡ $P < 0.001$, and § $P < 0.01$.

‖ NG vs NP: $P < 0.001$.

It would appear likely, based on prior biochemical studies, that both NG and NP activate guanylate cyclase through the formation of NO or related compounds [9–15]. It is evident from the present study that *in vitro* aorta and vena cava differ significantly in their responsiveness to the two agents. Thus, the binding, uptake, and/or processing of these drugs may be critical in their abilities to activate the guanylate cyclase system. Mechanisms by which NG and NP affect PGI_2 are unknown, but similar factors may play a role. Elucidating the mechanisms of action of these drugs, including their handling by vascular tissue from different anatomical sites, should lead to a better understanding of their therapeutic actions. The *in vitro* model for studying aorta or vena cava responsiveness may be useful for evaluation of derivatives with desirable selective circulatory effects.

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