Proc. Natl. Acad. Sci. USA
Vol. 74, No. 8, pp. 3203–3207, August 1977
Biochemistry

Nitric oxide activates guanylate cyclase and increases guanosine 3′:5′-cyclic monophosphate levels in various tissue preparations

(nitro compounds/adenosine 3′:5′-cyclic monophosphate/sodium nitroprusside/sodium azide/nitrogen oxides)

WILLIAM P. ARNOLD, CHANDRA K. MITTAL, SHOJI KATSUKI, AND FERID MURAD

Division of Clinical Pharmacology, Departments of Medicine, Pharmacology, and Anesthesiology, University of Virginia, Charlottesville, Virginia 22903

Communicated by Alfred Gilman, May 16, 1977

ABSTRACT Nitric oxide gas (NO) increased guanylate cyclase [GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2] activity in soluble and particulate preparations from various tissues. The effect was dose-dependent and was observed with all tissue preparations examined. The extent of activation was variable among different tissue preparations and was greatest (19- to 33-fold) with supernatant fractions of homogenates from liver, lung, tracheal smooth muscle, heart, kidney, cerebral cortex, and cerebellum. Smaller effects (5- to 14-fold) were observed with supernatant fractions from skeletal muscle, spleen, intestinal muscle, adrenal, and epididymal fat. Activation was also observed with partially purified preparations of guanylate cyclase. Activation of rat liver supernatant preparations was augmented slightly with reducing agents, decreased with some oxidizing agents, and greater in a nitrogen than in an oxygen atmosphere. After activation with NO, guanylate cyclase activity decreased with a half-life of 3–4 hr at 4° but re-exposure to NO resulted in reactivation of preparations. Sodium azide, sodium nitrite, hydroxylamine, and sodium nitroprusside also increased guanylate cyclase activity as reported previously. NO alone and in combination with these agents produced approximately the same degree of maximal activation, suggesting that all of these agents act through a similar mechanism. NO also increased the accumulation of cyclic GMP but not cyclic AMP in incubations of minces from various rat tissues. We propose that various nitro compounds and those capable of forming NO in incubations activate guanylate cyclase through a similar but undefined mechanism. These effects may explain the high activities of guanylate cyclase in certain tissues (e.g., lung and intestinal mucosa) that are exposed to environmental nitro compounds.

Guanylate cyclase [GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2], the enzyme that catalyzes the formation of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) from GTP, is activated by various agents including sodium azide, hydroxylamine, nitroglycerin, sodium nitrite, and sodium nitroprusside (1–7). The mechanism of this activation, however, has been unclear. Activation by sodium azide, hydroxylamine, and nitroglycerin has demonstrated tissue specificity (1–4), whereas activation by sodium nitroprusside is observed with most preparations (1). This has been thought to be due to the presence or absence of protein activators and inhibitors that can modify the effects of some of these agents (1, 2, 4, 8). A common feature of these compounds is that they either contain nitroso groups or are capable of forming nitroso compounds under certain conditions (1, 9–11). Recently, we demonstrated that nitric oxide gas (NO), generated by the addition of sodium nitrite to ferrous sulfate and sulfuric acid or obtained commercially, resulted in rapid activation of guanylate cyclase from supernatant fractions of bovine lung and tracheal smooth muscle (1). In this communication, we report the results of further investigation of this activation. NO activated all crude and partially purified guanylate cyclase preparations examined. It also increased cyclic GMP but not adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels in incubations of minces from various rat tissues.

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 150–250 g were decapitated. Tissues were rapidly removed, placed in cold 0.25 M sucrose/10 mM Tris·HCl buffer (pH 7.6), and homogenized in nine volumes of this solution by using a glass homogenizer and Teflon pestle at 2–4°. Bovine lung was obtained from a local abattoir, placed in cold saline within 10 min of sacrifice, and subsequently homogenized by the same technique as used with rat tissues. Homogenates were centrifuged at 105,000 × *g* for 60 min to obtain supernatant and particulate fractions. Adrenal homogenates were centrifuged at 15,000 × *g* for 60 min. Pellets were washed once with fresh medium, recentrifuged as described above, and suspended in a volume of buffer equal to that of the homogenates. Some supernatant preparations of guanylate cyclase were applied to DEAE-cellulose columns and eluted with a NaCl gradient as described (4, 8) or were applied to Sephadex G-100 columns (1.7 × 80 cm) that were eluted with 10 mM Tris·HCl buffer, pH 7.6/1 mM EDTA.

Guanylate cyclase activity was determined as described (1–4). Reaction mixtures in 10 × 75 mm tubes contained the enzyme preparation, 50 mM Tris·HCl buffer (pH 7.6), 10 mM theophylline, 15 mM creatine phosphate, creatine phosphokinase (120–130 units/mg) at 20 μg, and other compounds at the concentrations indicated. NO (Matheson Gas Products) was introduced into some reaction mixtures by venting the gas at a predetermined, constant flow rate from a polyethylene tube centered 1 cm above the reaction mixture. Flow rates were varied from 0.3 to 2.5 ml/min and volumes of NO added were determined by time of exposure. For most experiments, the flow rate was 1 ml/min with a 10-sec exposure. All tubes were flushed with a high flow of nitrogen for 5 sec before and after NO exposure to prevent the formation of nitrogen dioxide. Reactions were initiated by the addition of 4 mM $MnCl_2$/1 mM GTP in a final volume of 100 μl, incubated for 10 min at 37°, and terminated by the addition of 1 ml of 50 mM sodium acetate buffer (pH 4) to prevent nonezymatic formation of cyclic GMP (12) and heating at 90° for 3 min. Cyclic GMP formed was determined with the radioimmunoassay method of Steiner *et al.* (13) with some modification (14). With these assay conditions, formation of cyclic GMP was linear with incubation time and protein concentrations used. Unless indicated, values

Abbreviations: cyclic GMP, guanosine 3′:5′-cyclic monophosphate; NO, nitric oxide gas; cyclic AMP, adenosine 3′:5′-cyclic monophosphate.

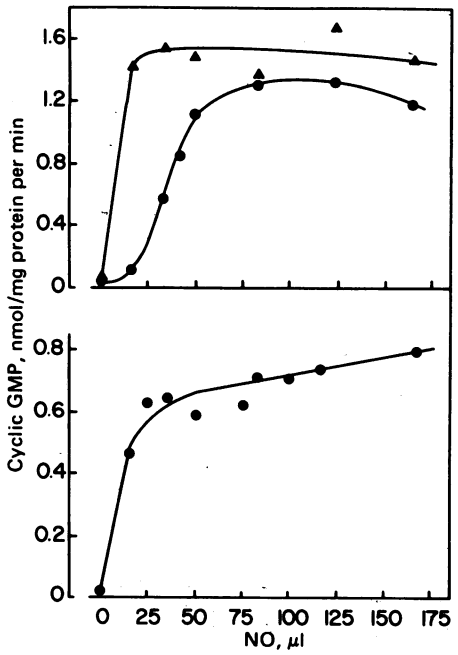

FIG. 1. Activation of guanylate cyclase from bovine lung and rat liver supernatant fractions. Soluble guanylate cyclase from (*Lower*) rat liver (70 μg of protein) (●) and (*Upper*) bovine lung (50 μg of protein) (●) and lung enzyme subjected to Sephadex G-100 chromatography (9 μg of protein) (▲) were incubated as described in *Materials and Methods* with increasing amounts of NO.

presented are means of duplicate or triplicate incubations from representative experiments.

Some rat tissues were placed in cold Krebs–Ringer bicarbonate solution containing glucose (1 mg/ml) and gassed with 95% O_2/5% CO_2. Tissue minces (0.3 × 0.3 mm) were prepared with a McIlwain tissue chopper and preincubated with fresh Krebs–Ringer bicarbonate solution for 30 min. Tissue incubations were conducted in 95% O_2/5% CO_2. To initiate incubations, we transferred minces to tubes (15 × 85 mm) containing 1 ml of fresh medium. All incubations were gassed with nitrogen, and some additionally with NO, for 10 sec as described above using a flow rate of NO of 2.5 ml/min. All samples were incubated for 3 min, terminated with 0.5 ml of 18% trichloroacetic acid, homogenized, and centrifuged. Supernatant fractions were extracted with ether and assayed for cyclic GMP and cyclic AMP by radioimmunoassay (13, 14) as described (3, 5, 14). Samples with low levels of cyclic nucleotides were acetylated prior to radioimmunoassay (15). Protein was determined with the method of Lowry *et al.* (16).

RESULTS

Stimulation of Guanylate Cyclase by NO. When supernatant fractions of rat liver and bovine lung homogenates were exposed to increasing volumes of NO, a dose-dependent increase in guanylate cyclase activity occurred (Fig. 1). In crude supernatant fractions, guanylate cyclase of rat liver homogenates was slightly more sensitive to NO than was that of bovine lung homogenates. Maximal activation of crude liver and lung enzymes occurred with 25 and 50 μl of NO, respectively. However, guanylate cyclase from lung, after passage through a Sephadex G-100 column, was maximally activated by 15 μl of NO. Assuming complete atmospheric mixing, a partition coefficient of 0.05, and even distribution in solution, addition of 25 μl of NO would result in a calculated concentration of the gas in solution of about 18 μM. Diffusion of NO into the reaction mixture did not seem limited by the surface area of the gas–liquid interface because activation of guanylate cyclase was not altered by shaking the mixture during gas exposure. With volumes of NO greater than 250 μl, stimulation was decreased (data not shown). In some experiments, NO was converted, at least in part, to nitrogen dioxide by adding nitric oxide to tubes containing 95% O_2/5% CO_2 rather than 100% nitrogen. In these experiments, some activation still occurred but was markedly reduced. Although the presence of nitrogen dioxide was obvious because of the appearance of a brown tint in the gas mixture, the extent of conversion of nitric oxide to nitrogen dioxide was not determined.

Table 1. Effect of NO on guanylate cyclase activity from various tissues

Tissue	Fraction	Cyclic GMP formed, pmol/mg protein per min −NO	+NO	Ratio +NO/−NO
Rat liver	S	22.1	674.2	30.5
	P	12.4	37.3	3.0
Bovine lung	S	111.7	3625.5	32.5
Bovine tracheal smooth muscle	S	8.9	297.1	33.4
	P	27.0	35.3	1.3
Rat heart	S	10.5	242.8	23.1
Rat kidney	S	51.6	975.9	18.9
Rat cerebral cortex	S	55.7	1122.6	20.2
	P	14.2	209.1	14.7
Rat cerebellum	S	23.7	784.4	33.1
	P	20.6	201.2	9.8
Rat skeletal muscle	S	6.1	84.0	13.8
Rat spleen	S	73.5	381.7	5.2
Rat small intestinal muscle	S	42.9	250.6	5.8
Rat adrenal	S	37.5	394.2	10.5
Rat epididymal fat	S	9.4	108.5	11.5
Rat liver	DEAE-cellulose	296.2	1406.3	4.7
Rat cerebral cortex	DEAE-cellulose	57.2	174.3	3.0
Bovine lung	Sephadex G-100	61.4	1672.1	27.2
Rat heart	Sephadex G-100	30.2	171.9	5.7

Supernatant (S) and particulate (P) preparations of guanylate cyclase and supernatant fractions partially purified on Sephadex G-100 or DEAE-cellulose were assayed as described in *Materials and Methods*. Where indicated, preparations were exposed to 165 μl of NO immediately before the addition of GTP and Mn^{2+}.

Tissue Specificity of Guanylate Cyclase Stimulation by NO. Table 1 summarizes the effect of NO with guanylate cyclase preparations from supernatant and particulate fractions from various tissues. Additional preparations of supernatant fractions were partially purified on either Sephadex G-100 or DEAE-cellulose columns prior to enzyme assay. NO markedly activated all tissue preparations examined except for the particulate enzyme from bovine tracheal smooth muscle in which a small effect (30%) was observed. These effects of NO are qualitatively similar to those observed with sodium nitroprusside activation of guanylate cyclase in these tissues (1). Sephadex G-100 or DEAE-cellulose chromatography of soluble guanylate cyclase from rat liver, heart, and cerebral cortex resulted in less activation with NO. Activation of partially purified prepara-

Table 2. Effect of NO and other compounds on soluble rat liver guanylate cyclase

Addition	Cyclic GMP formed (pmol/mg protein per min) −NO	%	+NO	Ratio +NO/−NO
None	51.0	(100)	817.8	16.0
EGTA, 0.1 mM	48.1	(94)	871.4	18.1
EDTA, 0.1 mM	46.6	(91)	832.2	17.9
EDTA	49.3	(97)	1050.4	21.3
Dithiothreitol	48.5	(95)	1299.8	26.8
Glutathione	47.0	(92)	1061.1	22.6
Ascorbate	48.8	(96)	1035.1	21.2
Dehydroascorbate	50.1	(98)	810.4	16.2
H_2O_2, 10 mM	46.5	(91)	649.2	14.0
Methylene blue, 0.1 mM	49.6	(97)	198.4	4.0
Methylene blue	57.8	(113)	159.3	2.8
Triton X-100, 1%	60.0	(118)	59.0	1.0
Ethanol	56.8	(111)	822.0	14.5
Phenol	49.0	(96)	728.7	14.9
KCN	52.2	(102)	864.7	16.6
$K_3Fe(CN)_6$	38.9	(76)	47.5	1.2
$K_4Fe(CN)_6$	35.2	(69)	709.5	20.2
Sodium nitroprusside 0.1 mM	283.6	(556)	683.6	2.4
Sodium nitroprusside	622.4	(1220)	607.4	1.0
$NaNO_2$	55.6	(109)	1000.3	18.0
$NaNO_2$, 10 mM	590.0	(1157)	850.9	1.4
NaN_3	346.6	(680)	935.5	2.7
NH_2OH	89.3	(175)	656.1	7.3
N_2 atmosphere	49.2	(96)	832.2	16.9

Soluble rat liver guanylate cyclase was assayed as described in *Materials and Methods*. Some incubations were exposed to 165 μl of NO gas. When present, other agents were 1 mM unless otherwise indicated. Some control and NO-treated incubations were conducted in a nitrogen atmosphere for the entire 10-min incubation. Values are means of duplicate or quadruplicate incubations.

tions from bovine lung was unaltered. Overnight dialysis at 4° of either the crude supernatant fraction from rat liver or the particulate fraction from bovine tracheal muscle did not alter the response of these preparations to NO. Activation of guanylate cyclase preparations first subjected to gel filtration, ion exchange chromatography, or dialysis suggested that there are no other requirements for NO activation. However, this remains to be established with highly purified preparations of guanylate cyclase. In analogous experiments, activation of liver guanylate cyclase with sodium azide was lost or markedly diminished with DEAE-cellulose chromatography but not with gel filtration or dialysis. This is due to the removal of a required protein activator factor (4, 8, 17).

Effects of Various Compounds on Guanylate Cyclase and Activation with NO. Activation of soluble guanylate cyclase from rat liver homogenates with NO was unaltered with EGTA or EDTA, suggesting that a free cation was not required (Table 2). Several reducing agents including dithiothreitol, glutathione, and ascorbic acid increased the effect of NO. Whereas H_2O_2 was slightly inhibitory, methylene blue, Triton X-100, and $K_3Fe(CN)_6$ markedly decreased the effect of NO. These agents produce similar effects on guanylate cyclase activation by sodium azide or sodium nitroprusside (1, 2, 8). Guanylate cyclase was also activated by sodium nitroprusside , sodium nitrite, sodium azide, and hydroxylamine as reported previously (1–4). Most of these agents alone were less effective than NO, and in

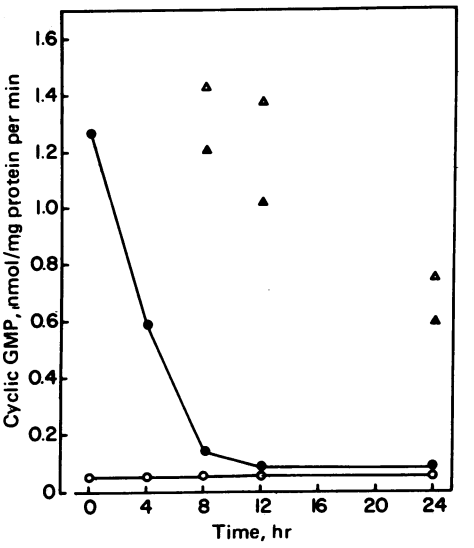

FIG. 2. Reversibility of soluble rat liver guanylate cyclase activation with NO and its reactivation. Soluble rat liver guanylate cyclase was exposed to maximally effective amounts of NO (250 μl). Activated and control enzyme were kept at 4°. At the times indicated, aliquots were reassayed for activity without and with exposure or re-exposure to NO. ○, Control enzyme; ●, enzyme exposed to NO at zero time; △, control enzyme exposed to NO at 8, 12, or 24 hr; ▲, activated enzyme re-exposed to NO at 8, 12, or 24 hr.

the presence of some of these agents NO produced further activation (Table 2). With NO plus either sodium nitroprusside or hydroxylamine, activation was somewhat less than with other combinations or NO alone. However, activation with several of these in the presence of NO agents was similar to that observed with NO alone. These observations suggest that the mechanism of activation by these diverse agents is identical because the stimulatory effects were not additive and were altered similarly by redox agents. Activation by NO was not affected when incubations were conducted in a nitrogen atmosphere. This is in contrast to activation with sodium azide which requires oxygen (8).

Reversibility of NO Activation. Soluble rat liver guanylate cyclase was maintained at 4° with and without prior exposure to NO (Fig. 2). At various times, native and NO-activated enzyme were assayed for activity without and with exposure to NO. Basal enzyme activity was quite constant for 24 hr at 4°. However, the activation induced by the exposure of the control preparation to NO declined over 24 hr. The activity of the preparation exposed to nitric oxide at zero time declined with a half-life at 4° of about 3–4 hr. This preparation could be reactivated by a second exposure to NO, and the magnitude of that response was slightly less than the activated control preparation. These experiments indicate that nitric oxide activation is slowly reversible at 4° and that the enzyme can be reactivated. However, the maximal effect is somewhat labile in that the total activity achievable with NO activation declines with time. Similar results were observed with control and NO-activated enzyme that had been dialyzed at 4° overnight and exposed to NO just before reassay (data not shown).

Effects of NO on Cyclic GMP Levels in Incubations of Tissue Minces. Some incubations of tissue minces were exposed to 415 μl of gas (calculated concentration, 75 μM). NO increased cyclic GMP levels in all incubations (tissue plus medium) examined (Table 3). All increases in cyclic GMP were significant and greater than 6-fold with the exception of that in the cerebellum which was less than doubled ($P < 0.05$). Cyclic AMP

Table 3. Effect of NO on cyclic GMP and cyclic AMP levels in rat tissues

Tissue	Cyclic GMP, pmol/mg protein		Cyclic AMP, pmol/mg protein	
	Control	NO	Control	NO
Liver	0.03 ± 0.002	0.18 ± 0.05	1.04 ± 0.08	1.02 ± 0.04
Lung	0.19 ± 0.01	1.71 ± 0.45	17.85 ± 1.56	16.25 ± 1.30
Heart	0.06 ± 0.007	2.10 ± 0.48	2.46 ± 0.18	2.58 ± 0.15
Kidney cortex	0.08 ± 0.02	1.65 ± 0.12	4.27 ± 0.34	3.99 ± 0.29
Cerebral cortex	0.40 ± 0.07	2.51 ± 0.27	5.54 ± 0.66	5.29 ± 0.23
Cerebellum	3.69 ± 0.29	5.97 ± 0.90	15.72 ± 0.84	15.33 ± 2.87

Rat tissue minces (3–20 mg of protein) were incubated as described in *Materials and Methods* without and with exposure to 415 μl of NO. Values are means ±SEM of triplicate incubations.

levels were not altered by NO. However, other incubation times and NO concentrations were not examined. Sodium azide, hydroxylamine, sodium nitrite, nitroglycerin, and sodium nitroprusside increased cyclic GMP levels without altering cyclic AMP accumulation in preparations of liver, cerebral cortex, cerebellum, and tracheal smooth muscle (3, 5, 18). Some of these agents, however, increased both cyclic AMP and cyclic GMP levels in polymorphonuclear leukocyte preparations (data not shown).

DISCUSSION

Sodium azide, sodium nitrite, hydroxylamine, sodium nitroprusside, and nitroglycerin increase guanylate cyclase activity and cyclic GMP accumulation in various cell-free and intact-cell preparations (1–8, 17–21). Recently, nitrosamines (22), streptozotocin, and 1-methyl-1-nitrosourea (23) have also been reported to activate guanylate cyclase and/or increase cyclic GMP levels in several tissues.

Activation of guanylate cyclase with sodium azide is tissue specific due to the requirement for protein activator factors (1–4, 6–8, 17–21) and the effects of protein inhibitors (2). Guanylate cyclase in crude or partially purified preparations that are unresponsive to sodium azide can be activated with azide after the addition of activator protein preparations, catalase, peroxidase, or cytochrome b_2 (1, 2, 4, 7, 8, 17, 20, 21). In contrast, most guanylate cyclase preparations can be activated with sodium nitroprusside and no requirement for such protein factors has been observed (1). We have recently observed that NO gas generated from $NaNO_2$ in the presence of $FeSO_4$ and H_2SO_4 or obtained commercially markedly activates lung and tracheal muscle guanylate cyclase (1).

Because all of these activating agents can provide a source of NO or be converted to oxides of nitrogen with the appropriate conditions (1, 9–11), we investigated the effect of NO on guanylate cyclase activity and cyclic GMP accumulation in various cell-free and intact-cell preparations. As little as 15 μl of NO activated soluble guanylate cyclase from lung and liver homogenates (Fig. 1); 25–50 μl of NO was maximally effective with crude guanylate cyclase preparations and amounts greater than 250 μl were less effective. Assuming that the added gas was distributed uniformly and according to its partition coefficient and classical gas laws, these amounts should theoretically result in concentrations of NO in the incubation medium of 10–180 μM. A maximally effective amount of NO (165 μl) activated all guanylate cyclase preparations examined. Activation was variable with different tissue preparations and ranged from 3- to 33-fold. However, very small effects were observed with particulate preparations from tracheal smooth muscle. Because all preparations of crude, partially purified, and dialyzed enzyme were activated, it appears that NO does not require protein activators, small molecules, or other factors needed for sodium azide activation (1, 2, 4, 8, 17, 20, 21). This apparent lack of tissue specificity with NO activation was also observed with sodium nitroprusside activation of guanylate cyclase (1). NO activation was decreased somewhat with DEAE-cellulose or Sephadex G-100 chromatography of liver, heart, and cerebral cortex but not lung preparations (Table 1). Examination of the effects of NO on highly purified preparations of guanylate cyclase may establish whether NO serves to activate the enzyme directly or through interaction with other factors.

Reducing agents such as dithiothreitol, glutathione, and ascorbic acid increased the effect of NO. Various oxidizing agents either had no effect or markedly decreased activation by NO. Activation was similar in air and nitrogen atmospheres and was decreased in an atmosphere of 95% O_2/5% CO_2. The effects of oxidizing agents and oxygen are probably due to the conversion of NO to less effective higher oxides of nitrogen such as nitrogen dioxide. In contrast, reducing agents may prevent the oxidation of NO. However, other effects of oxidizing and reducing agents on native and activated guanylate cyclase cannot be excluded. These effects of redox agents upon the formation and/or activity of nitric oxide may explain several reports that oxygen, H_2O_2, methylene blue, and ascorbate have altered cyclic GMP accumulation and/or guanylate cyclase activity (24, 25).

Guanylate cyclase in the supernatant fraction of rat liver homogenates is activated to a lesser degree with sodium nitroprusside, sodium nitrite, sodium azide, and hydroxylamine (1–4, 6, 8, 17, 21). In the presence of some of these activators, NO resulted in a further increase in guanylate cyclase activity that was comparable to the activity observed with NO alone (Table 2). These observations are consistent with our hypothesis that activation of guanylate cyclase by these and other agents (22, 23) is due to the formation of NO in incubations.

Activation with NO was reversible at 4° with a half-life of 3–4 hr. Re-exposure to NO resulted in reactivation of the preparation. The requirements, if any, for this decrease in activity of activated enzyme were not examined. However, as with NO activation, free cations and small molecules were not required because activation and reversal were observed with dialyzed preparations.

NO also increased cyclic GMP accumulation in incubations of various tissue minces without altering cyclic AMP levels. Because sodium azide activation of guanylate cyclase alters the properties of the enzyme (2, 8, 17) and permits guanylate cyclase to catalyze the formation of cyclic AMP from ATP (21), one might expect increases in cyclic AMP with NO exposure. The absence of changes in cyclic AMP levels with NO suggests that either cyclic AMP formation by activated guanylate cyclase does not occur in intact tissues or our incubation conditions were not suitable. Sodium azide, sodium nitroprusside, nitroglycerin, and hydroxylamine also increase cyclic GMP levels in incubations of liver, cerebral cortex, cerebellum, and tracheal muscle without altering cyclic AMP levels (3, 5, 18). However, sodium azide, sodium nitroprusside, and hydroxylamine increased cyclic AMP and cyclic GMP levels 5- to 20-fold in incubations of human polymorphonuclear leukocytes preparations (F. Murad and R. T. Curnow, unpublished data).

The physiological and toxicological implications of these observations lead to interesting speculation regarding guanylate cyclase regulation by certain environmental, dietary, and

pharmacological agents because some of these compounds contain nitro groups or are capable of releasing or forming NO. For example, the high activities of guanylate cyclase in lung and intestinal mucosa may result from airborne or dietary factors. In addition, the effects of some nitro-containing compounds on smooth muscle relaxation and tissue proliferation may be associated with guanylate cyclase activation and cyclic GMP accumulation (5, 22). Further studies are required to test these hypotheses and other speculations discussed above.

In summary, NO activation of guanylate cyclase appears to be a novel mechanism of enzyme regulation that may also be involved in the regulation of other enzymes and systems.

The authors thank Robert Aldred, Bing Chang, and Joanne Holmes for their technical assistance and Dr. Robert M. Epstein for his assistance in calculating the theoretical concentration of NO. These studies were supported by grants from the National Institutes of Health (AM 15316, HL 18260, and AM 17042). W.P.A., a fellow in Clinical Pharmacology and Anesthesiology, was supported by a National Institutes of Health Clinical Pharmacology Training Grant GM 10 and is currently a recipient of a Pharmaceutical Manufacturers Association Foundation Fellowship. F.M. is a recipient of a National Institutes of Health Research Career Development Award AM 70456.

1. Katsuki, S., Arnold, W., Mittal, C. & Murad, F. (1977) *J. Cyclic Nucleotide Res.* **3**, 23–35.
2. Kimura, H., Mittal, C. K. & Murad, F. (1975) *J. Biol. Chem.* **250**, 8016–8022.
3. Kimura, H., Mittal, C. K. & Murad, F. (1975) *Nature* **257**, 700–702.
4. Mittal, C. K., Kimura, H. & Murad, F. (1975) *J. Cyclic Nucleotide Res.* **1**, 261–269.
5. Katsuki, S. & Murad, F. (1977) *Mol. Pharmacol.* **13**, 330–341.
6. Katsuki, S., Arnold, W. P., Mittal, C. K. & Murad, F. (1977) *Clin. Res.* **25**, 58a.
7. Deguchi, T. (1977) *J. Biol. Chem.* **252**, 596–601.
8. Mittal, C. K., Kimura, H. & Murad, F. (1977) *J. Biol. Chem.* **252**, 4384–4390.
9. Keilin, D. & Hartree, E. F. (1954) *Nature* **173**, 720–723.
10. Woolum, J. C. & Commoner, B. (1970) *Biochim. Biophys. Acta* **201**, 131–140.
11. Theorell, H. & Ehrenberg, A. (1952) *Arch. Biochem. Biophys.* **41**, 462–474.
12. Kimura, H. & Murad, F. (1974) *J. Biol. Chem.* **249**, 329–331.
13. Steiner, A. L., Parker, C. W. & Kipnis, D. M. (1972) *J. Biol. Chem.* **247**, 1106–1113.
14. Kimura, H., Thomas, E. & Murad, F. (1974) *Biochim. Biophys. Acta* **343**, 519–528.
15. Harper, J. F. & Brooker, G. (1975) *J. Cyclic Nucleotide Res.* **1**, 207–218.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
17. Kimura, H., Mittal, C. K. & Murad, F. (1976) *J. Biol. Chem.* **251**, 7769–7773.
18. Katsuki, S. & Murad, F. (1977) *Clin. Res.* **25**, 32a.
19. DeRubertis, F. R. & Craven, P. A. (1976) *J. Biol. Chem.* **251**, 4651–4658.
20. Miki, N., Nagano, M. & Kuriyama, K. (1976) *Biochem. Biophys. Res. Commun.* **72**, 952–959.
21. Mittal, C. K. & Murad, F. (1977) *J. Biol. Chem.* **252**, 3136–3140.
22. DeRubertis, F. R. & Craven, P. A. (1976) *Science* **193**, 897–899.
23. Vesely, D. L., Rovere, L. E. & Levey, G. S. (1977) *Cancer Res.* **37**, 28–31.
24. Clyman, R. I., Blacksin, A. S., Manganiello, V. C. & Vaughan, M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3883–3887.
25. White, A. A., Crawford, K. M., Patt, C. S. & Lad, P. J. (1976) *J. Biol. Chem.* **251**, 7304–7312.