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β-Mercaptoguanidine derivatives – new class of potential NO-generating compounds

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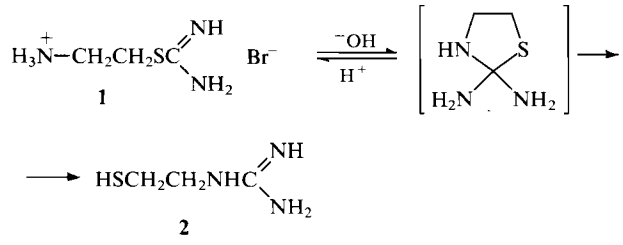
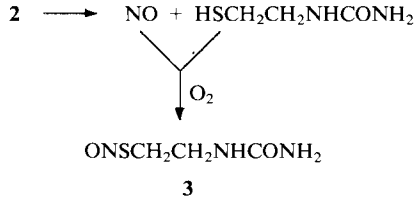
β-Mercaptoguanidine derivatives generate nitric oxide under oxidation *in vitro* and *in vivo*, activate soluble guanylate cyclase and diminish arterial blood pressure.

Endogenous nitric oxide (NO) formed by oxidation of L-arginine plays an important role in the control of vascular tone.[1] The mechanism of NO action is connected with the activation of soluble guanylate cyclase, which catalyses the synthesis of guanosine 3′,5′-cyclic monophosphate. Increasing the level of this nucleotide decreases the tone of vascular smooth muscle and leads finally to vasodilation.[2] Owing to this, the synthesis of NO-generating compounds has caused significant interest with the aim to creating new antihypertensive drugs.[3] Taking into account the fact that nitric oxide is the active basis of such known drugs as nitroglycerol, nitrosorbid, sodium nitroprusside, molsidomine *etc.*, the main effort in research is directed to the synthesis of compounds which have NO- or NO_2-containing fragments in evident or disguised forms, for example, nitroesters and *S*-nitrosothiols. The latter group are especially interesting since the nitrosothiols are probably the carriers of NO to guanylate-cyclase heme.[4] However, the instability of compounds of this type makes difficult the search for drugs based on the synthesis of SNO-derivatives.[5] Our interest lies in attempts to combine the approaches based on the search for systems which are capable of forming NO after oxidation and on the preparation of substituted *S*-nitrosothiols as intermediates (*in vitro* and *in vivo*).

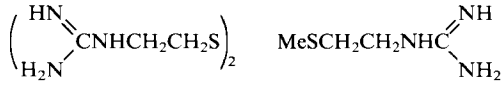
In our opinion, a promising object for such investigation is *S*-(β-aminoethyl)guanidine hydrobromide **1**, which transforms (pH ~7) readily to β-mercaptoethylguanidine **2**.[6,7]



In the present work, the quantitative determination of the relationship between compounds **1** and **2** is provided by polarographic methods in buffer solution at different pH values using the value of the limiting anodic current of SH-group oxidation [compound **1** is electrochemically inactive in an Hg-drop electrode over the range of potentials +0.2 to −1.7 V (SCE)]. The limiting anodic current, which is absent at pH 1–2, is fixed at pH 3, increased to pH ~7 and then kept constant on further increase of pH. This indicates that transformation **1**→**2** is complete under these conditions and shows the possibility of this conversion *in vivo*. It is possible to suppose that upon oxidation of the guanidine moiety of **2** (as for the oxidation of arginine[8]) NO-release occurs followed by NO uptake by an 'intramolecular trap': the SH-group, with the formation of *S*-nitrosothiol **3**:



Disulfide **4**, synthesized according to ref. 9 is capable of behaving similarly. The secondary process (formation of S–NO compound) is excluded, of course, for β-methylmercaptoguanidine **5**. Thus, **5** synthesized according to ref. 10 is a model compound for examination of the influence of S-nitrosation upon biological activity.



To comfirm the above suggestions, the ability of compounds **2**, **4** and **5** (in comparison with L-arginine **6**) to activate the soluble platelet guanylate cyclase was estimated (for details of the procedure see ref. 11). It was found that the compounds investigated form NO under oxidation by the same mechanism as for arginine **6**. This oxidation proceeds with participation of a specific enzyme — NO-synthase. Unambigous proof of this is provided by the inhibition of the activation process in the presence of the inhibitor of this enzyme — ω-*N*-methylarginine.

The results of the investigation, presented in Table 1, testify that the presence of an SH-group or disulfide link (which is capable of being a trap) provides far greater activation of enzyme as compared with **5** and **6**. It is necessary to note that

Table 1 The effect of β-mercaptoguanidine derivatives on the activity of human platelet soluble guanylate cyclase.

	Degree of enzyme activation[a] Concentration of compound/mol dm^{-3}			
Compound	10^{-6}	10^{-5}	10^{-4}	5×10^{-4}
6	1.08±0.06	1.08±0.15	1.20±0.08	1.16±0.05
2	1.72±0.15	2.04±0.18	2.84±0.30	1.80±0.18
4	2.52±0.18	5.80±0.25	6.08±0.32	3.20±0.24
5	–	–	1.19±0.03	–

[a] It is a well-known phenomenon that an increase in substrate concentration can lead to an initial increase, followed by a decrease, in the degree of guanylate cyclase activation (bell-like dependence of activation from concentration).[11]

Table 2 The effect of β-mercaptoguanidine derivatives on the arterial pressure of rats with reno-vascular hypertension.

Compound	dose (mg kg^{-1}, i.v.)	decrease in AP (mmHg)	LD_{50} (mg kg^{-1}, i.v.)
6	1.0	5.0±1.3	>200
	5.0	6.5±0.9	
	10.0	12.0±0.8	
2	0.1	4.7±2.6	
	0.5	7.2±1.2	133±2.5
	1.0	13.2±1.4	
	5.0	31.6±3.7	
4	0.1	6.0±1.3	82±3.5
	0.5	12.5±1.9	
	1.0	17.5±2.1	
	5.0	23.0±3.8	
5	0.1	4.0±3.1	58±3.6
	0.5	5.3±1.4	
	1.0	10.5±2.5	
	5.0	21.5±3.3	

the efficiency of disulfide **4** is superior to that of **2**. Incomplete rearrangement **1**→**2** under these conditions probably results.

The *in vivo* antihypertensive effect of compounds was studied on anaesthetized rats with reno-vascular hypertension. Compounds were administered intravenously. The effect of each dose was determined on 4–6 rats.

As shown in Table 2, all compounds lower arterial pressure (AP) in a dose-dependent fashion. At tested doses the antihypertensive effect of **2**, **4** and **5** are more pronounced as compared with L-arginine. The dose 10 mg kg^{-1} for L-arginine **6** is as effective as 0.5 mg kg^{-1} for **4** and 1.0 mg kg^{-1} for **2** and **5**. At dose 5 mg kg^{-1} the decrease of AP caused by **2**, **4** and **5** is 2–3 times higher than lowering of AP induced by L-arginine at dose 10 mg kg^{-1}.

It is evident that *in vivo* the difference between the efficiencies of **4** and **2** as compared with **5** is less than that predicted from data on the activation of guanylate cyclase. Nevertheless, the common trend in the change in activity of these compounds is the same. It is possible that this resemblance of activity of **2** and **4** with **5** *in vivo* is a result of enzyme demethylation of the S–Me-group in mammalian organisms.

Thus, the data obtained show that compounds having a guanidine fragment in their structures are able to produce nitric oxide after transformation and that the presence of an SH-group leads to an increase in biological activity. The results obtained confirm that it is expedient to continue the search for NO-generating compounds among guanidine derivatives containing thiol groups as substituents.

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