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SOME ANTIHYPERTENSIVE DRUGS CLONIDINE (CLOFELIN) AND GUANFACINE – NEW NITRIC OXIDE DONORS

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The presence of in vitro NO-donor characteristics in the antihypertensive drugs clonidine and guanfacine, the structure of which contains a guanidine grouping that relates these drugs to arginine (the main substrate of the enzyme NO-synthase, producing NO in vivo) was established by chemical oxidation of the drugs followed by polarographic detection of the produced nitric oxide in the form of nitroprusside ion. It was shown that under the investigated conditions in vitro the NO-donor activity of clonidine and guanfacine is comparable in magnitude with the NO-donor activity of arginine. It was also found that guanfacine and clonidine (the latter in the presence of NAD^+) activate the soluble guanidatecyclase enzyme, which is a characteristic indication of NO donors. It is suggested on the basis of the obtained data that clonidine and guanfacine can undergo enzymatic oxidation to NO in vivo and that the pharmacological action of clonidine and guanfacine may be due to some extent to the NO-donor characteristics of these drugs.

Keywords: arginine, guanfacine, clonidine (clofelin), nitroprusside anion, NO, isolation, polarographic detection, oxidation.

The discovery of the significant biological role of nitric oxide in the function of the living organism brings about the need to examine new aspects of the physicochemical action of various familiar drugs. Today there is no longer any doubt that the biological activity of nitroglycerol, nitrosorbide, and other nitrates is due directly to their ability to release nitric oxide *in vivo* [1]. It has also been reliably established that it is the NO-donor ability of the antihypertensive drug molsidomine that gives rise to its pharmacological action. The biological activity of nitric oxide is based on its participation in the regulation of the tone of blood vessels, inhibition of the aggregation of thrombocytes, dilation of the smooth muscles of blood vessels and the gastrointestinal tract (GIT), regulation of the respiratory system, GIT, urinogenital system, stabilization of cellular immunity, etc. [2-8]. It is well known that nitric oxide is formed in the organism by the oxidation of L-arginine with the participation of the NO-synthase (NOS) enzymes, and the functions of the NO correlate with the three distinct forms of NOS – the constitutive endothelial form (cNOS), the neuronal isoform (nNOS), and the inducible form (iNOS), the actions of which include respectively regulation of the relaxation of the smooth musculature of the vessels, the neurotransmitter characteristics of NO, and the activation of macrophagous cells (cytotoxic activity) [9-13]. The degree of specificity of these enzyme systems is probably not directed absolutely at *L*-arginine, and it is well known that NO-donor activity is characteristic of a large number of guanidine and guanidine-like systems, while the characteristics of these compounds as generators of NO show up not only *in*

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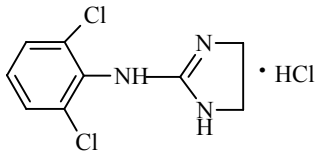
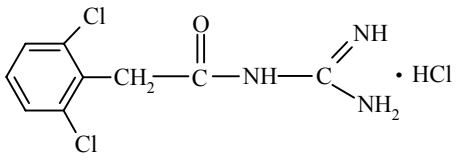
vitro but also *in vivo* [14]. One cannot fail to mention also that nitric oxide during the oxidation of organic molecules can, in principle, be formed not only with the participation of the isoforms of the NOS but also during the action of other oxidizing enzymes. In one way or another a new examination of familiar biologically active compounds whose structural characteristics make it possible to suppose that they are capable of acting as generators of nitric oxide is both timely and expedient. Recently [15], we showed that the well-known psychotropic drugs sydnophen and sydnocarb are extremely active NO donors. The present paper is devoted to a study of the NO-donor characteristics of guanfacine and clonidine (clofelin), which are widely used in medical practise. The starting point for such an investigation is the presence in the structures of these compounds of a guanidine fragment (open in guanfacine and cyclic in clonidine), suggesting that the oxidative degradation of these drugs can lead to nitric oxide.



Clonidine

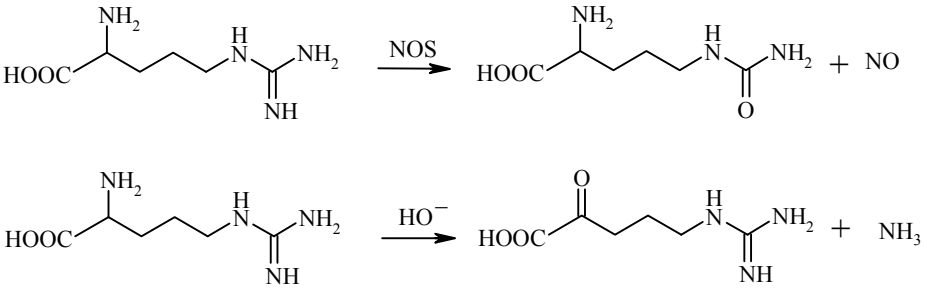


Guanfacine

Clonidine and guanfacine belong to the group of drugs that affect the vasomotory centers [16]. By passing through the hematoencephalic barrier, both drugs stimulate the α_2-adrenoreceptors of the vasomotory centers and reduce the flow of sympathetic pulses from the central nervous system and reduce the release of noradrenaline from the nerve endings, having to some degree sympatholytic action. Thus, the main result of the administration of both drugs is a hypotensive effect. In the case of clonidine a sustained hypotensive effect may be preceded by a brief hypertensive effect caused by excitation of the peripheral α-adrenoreceptors.

The reduction of central adrenergic activity is put down to the ability of clonidine to remove the somatovegetative manifestations of opiate and alcoholic abstinence and, in particular, to reduce phobia. Clonidine also has a sedative and analgesic effect regardless of the abstinence syndrome.

First a few words about an investigation devoted to the chemical oxidation of *L*-arginine by potassium ferricyanide [17], in which it was established that the predominant oxidation product is the α-keto acid, i.e., in contrast to *in vivo* enzymatic oxidation the guanidine group is not affected under the conditions of chemical oxidation. Oxidation takes place mainly at the α-carbon atom, the amino group is eliminated in the form of NH_3, and the formation of NO is not detected.



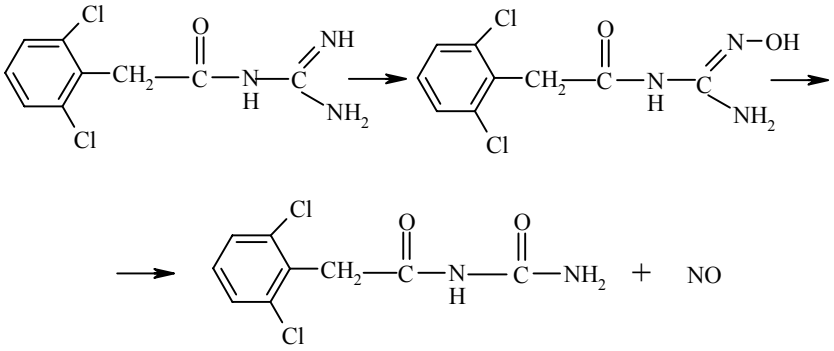
These results cast doubt on the correctness of using prediction of *in vivo* NO-donor activity from chemical oxidation. In the present work, therefore, the results obtained by this method for clonidine and guanfacine were compared with the results for *L*-arginine.

It was found that all three compounds are capable of releasing NO during chemical oxidation, but differences were found between, and arginine occupies an intermediate position between guanfacine and clonidine in NO-donor activity (Tables 1-3). As follows from Table 1, guanfacine releases NO fairly readily

during oxidation. For clonidine it was not immediately possible to determine the conditions for the occurrence of such a process. Experiments on the oxidation of clonidine were conducted with variation of all the conditions: Temperature (15-90°C); time (15 min to three months); pH 6-14; oxidizing agents (potassium ferricyanide, hydrogen peroxide, atmospheric oxygen, hemin, potassium persulfate); the concentration of the substance being oxidized (10^{-4}-$5\cdot10^{-3}$ M) and of the oxidizing agent (from a 10-fold to a 1000-fold excess of the compound being oxidized).

It is known [18] that a series of physiological functions of nitric oxide in the living organism are mediated by the activation of an enzyme – soluble guanylate cyclase (sGC) – and by the accumulation of cGMP. It is therefore natural to suppose that the compounds that are NO donors must give rise to some degree or other of activation of the sGC and accumulation of cGMP (depending on the amount of nitric oxide released). it was found that both clonidine and guanfacine cause activation of this enzyme independent of the concentration of the drug, and in accordance with the results of chemical oxidation the degree of expression of the activation of sGC during the action of guanfacine is higher (Table 4).

It can be supposed the oxidation of guanfacine takes place largely by the same mechanism as the oxidation of *L*-arginine, and the process can be summarized by a similar scheme:



A significant complication for clonidine is the presence of the cyclic imidazoline system. The hypothetical scheme for the oxidation of this drug looks as follows:

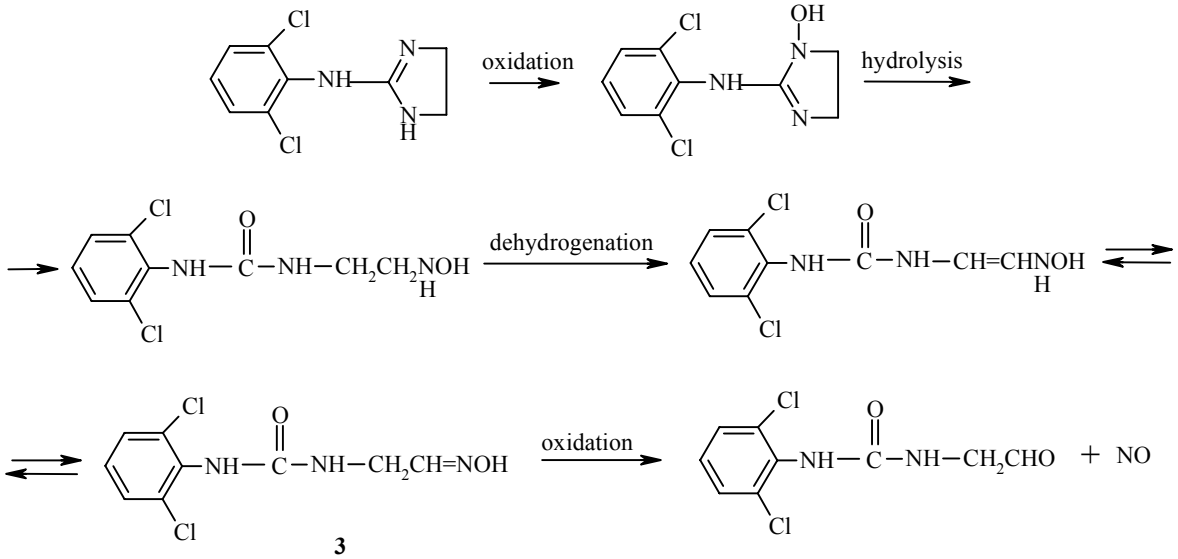


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TABLE 1. The Yield of the Nitroprusside (NP) Anion after Oxidation of Guanfacine, Clonidine, and *L*-Arginine at 80°C

Oxidant	Alkalinity	Time, h	Yield of NP, %, for:		
			Guanfacine	Clonidine	Arginine
$K_3[Fe(CN)_6]$, 3.5–10-fold.	Borate buffer pH 10	3.5	5	0	0
	0.12 M KOH	8.5	—	0.2	—
H_2O_2, init. conc. 1.4 M	1 M KOH	20	—	2.2	—

If this scheme is accepted, the key stage of the process is the dehydrogenation stage. The oxime **3** is then formed and is transformed according to the familiar scheme for the oxidation of oximes [19] into the corresponding carbonyl compound and nitric oxide. Since the medium in which the ability of the compounds to activate sGC was being investigated contained various enzymes, including dehydrogenase, it seemed expedient to accelerate the dehydrogenation process by adding the coenzyme NAD^+, capable of entering into reversible oxidation–reduction reactions with hydride-ion transfer. It was found that the addition of NAD^+ at the rate of 10^{-7} M increased the activation of sGC by clonidine by 22% and at 10^{-6} M by 90%, which to some extent confirms the ideas expressed above.

Thus, the reliability of the method employed in the present work to predict the NO-donor activity of a compound under the conditions of the living organism is supported by the results obtained during the chemical oxidation both of arginine and of guanfacine and clonidine. The data in Tables 1-3 for arginine demonstrate clearly the difference between enzymatic oxidation *in vivo* and chemical oxidation and explain the admissibility of the use of "harsh" conditions (increased temperature and oxidizing agents with a high oxidation potential, pH values far from neutral). In addition, these data explain the observations described in [17]; since nothing was yet known about endogenous NO, its formation with a low yield was not noticed.

TABLE 2. The Yield of the Nitroprusside (NP) Anion after the Oxidation of Clonidine and Arginine at Room Temperature for 3 Months (21.01-27.04)

Oxidant	Alkalinity	Yield of NP, %, for:	
		Clonidine	Arginine
Hemin	Unbuffered solution	0.2	0.5
	Borate buffer, pH 9.18	0.1	0.2
$K_3[Fe(CN)_6]$, 10-fold ratio	Unbuffered solution	3	—
	Borate buffer, pH 9.18	1.5	—
H_2O_2, init. ratio 10-fold	Unbuffered solution	0.4	—
	Borate buffer, pH 9.18	0.1	—

TABLE 3. The Yield of Nitroprusside (NP) Anion after the Oxidation of Clonidine and Arginine at Room Temperature for 3 Months (30.04-27.07)

Oxidant	Alkalinity	Yield of NP, %, for:	
		Clonidine	Arginine
$K_3[Fe(CN)_6]$, equimolar	Unbuffered solution	2.5	2.5
	Borate buffer, pH 9.2	4	4
H_2O_2, init. conc.0.25 M	Unbuffered solution	0	—
	Borate buffer, pH 9.2	1.8	8

TABLE 4. The Effect of Clonidine and Guanfacine on the Activation of sGC

Tested system	Activation of sGC at concentration of investigated drug			
	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
Clonidine	—	1.0	.9	1.3
Clonidine + NAD^+ (10^{-7} M)	—	—	1.1	—
Clonidine + NAD^+ (10^{-6} M)	—	—	1,7	—
Guanfacine	1.7	1.8	1.4	1.2
Guanfacine + NAD^+ (10^{-6} M)	1.3	1.4	—	—

In conclusion it is necessary to mention the unknown fact that the drugs guanfacine and clonidine, widely used in medicine, are nitric oxide donors and are capable of activating the enzyme soluble guanylatecyclase can certainly not be regarded as grounds for the reexamination of established ideas about the mechanism of the action of these drugs. At the same time the discovered new data deserve the special attention of pharmacologists with respect to the specific investigation of the reasons for the pharmacological actions of these drugs, possessing high antihypertensive activity.

EXPERIMENTAL

Chemical Oxidation and Detection of NO by Polarography. The oxidation of the investigated substances was conducted at concentrations of $2 \cdot 10^{-4}$-$1 \cdot 10^{-3}$ M. The NO was detected by polarography after it had been combined in the form of the nitroprusside ion $[Fe(CN)_5NO]^{2-}$ using the procedure described in [20, 21].

Biochemical Tests. The effect of the compounds on the activity of sGC was studied on a sample of the enzyme obtained from human thrombocytes according to [22]. A suspension of the washed thrombocytes in 50 mM Tris-HCl-buffer, pH 7.6, containing 0.2 mM of dithiotreitol, was subjected to ultrasonic treatment on an MSE 5-78 ultrasonic disintegrator (UK) for 20 sec at 2°C and was then centrifuged for 1 h at 105 000 d. The supernatant liquid was used as sample of guanylate cyclase. The activity of the sGC was determine according to [23]. The samples (final volume 150 μliter) contained 50 mM of Tris-HCl-buffer, pH 7.6, 1 mM of HTF, 4 mM of creatine phosphate, 20 μg (120-160 units) of creatine phosphokinase, 10 nM of theophylline, 105 000 d (15-20 μg as protein) of supernatant, and other additives where necessary. The investigated compounds were used at concentrations in the range of 10^{-7}-10^{-4} M.

The amount of cHMF formed in the enzymatic reaction (15 min at 37°C) was determined by an immunoenzyme method using a set of reagents from Bioimmunogen (Russia) for the determination of the cHMF.

The protein was determined by the method of Bradford [24]. The HTF sodium salt (Fluka, Switzerland) was used. The other reagents were from Sigma, USA.

REFERENCES

1. V. G. Granik, S. Yu. Ryabova, and N. B. Grigor'ev, *Usp. Khim.*, **65**, 792 (1997).
2. S. Moncada and E. A. Higgs, *Eur. J. Clin. Invest.*, **21** (4), 361 (1991).
3. S. Moncada, R. M. J. Palmer, and E. A. Higgs, *Pharmacol. Rev.*, **43**, 109 (1991).
4. J. S. Stamler, D. J. Singel, and J. Loscalzo, *Science*, **258**, 1898 (1992).
5. C. Nathan, *FASEB J.*, **6**, 3051 (1992).

6. F. Murad, *Neurotransmissions*, **10**, No. 2, 1 (1994).
7. B. Brune, S. Dimmeler, L. Molina y Vedia, and E. G. Lapetina, *Life Sci.*, **54**, 61 (1994).
8. R. M. Clancy and S. B. Abramson, *Proc. Soc. Exp. Biol. Med.*, **210**, 93 (1995).
9. O. W. Griffith and D. J. Stuehr, *Ann. Rev. Physiol.*, **57**, 707 (1995).
10. C. Nathan and Q. Xie, *J. Biol. Chem.*, **269**, 13725 (1994).
11. M. A. Marietta, *J. Biol. Chem.*, **268**, 12231 (1993).
12. D. J. Stuehr, H. M. Abu-Soud, D. L. Rousseau, P. L. Feldman, and J. Wang, *Adv. Pharmacol.*, **34**, 207 (1995).
13. D. A. Wink, M. B. Grisham, J. B. Mitchell, and P. C. Ford, *Methods. Enzymol.*, **268**, 12 (1996).
14. V. G. Granik, N. B. Grigoriev, L. H. Vinograd, I. S. Severina, M. D. Mashkovskyi, V. B. Nikitin, G. N. Engalycheva, M. A. Kalinkina, and O. G. Busigina, *Mendeleev Commun.*, 161 (1996).
15. V. I. Levina, N. B. Grigor'ev, and V. G. Granik, *Khim. Geterotsikl. Soedin.*, in the press.
16. M. D. Mashkovskii, *Drugs* [in Russian], Meditsina, Moscow (1993), Vol. 1.
17. D. Laloo and M. K. Mahanti, *Pol. J. Chem.*, **60**, 589 (1986).
18. I. S. Severina, *Biokhimiya*, **63**, 939 (1998).
19. L. N. Koikov, N. V. Alexeeva, E. A. Lisitza, E. S. Krichevsky, N. B. Grigoryev, A. V. Danilov, I. S. Severina, N. V. Pyatakova, and V. G. Granik, *Mendeleev Commun.*, 165 (1998).
20. V. I. Levina, D. A. Grigor'ev, and N. B. Grigor'ev, *Khim.-Farm. Zh.*, No. 8, 55 (1995).
21. V. I. Levina, A. V. Danilov, and N. B. Grigor'ev, *Khim.-Farm. Zh.*, No. 4, 53 (1998).
22. Yu. Yu. Chirkov, I. A. Tyshchuk, N. N. Belushkina, and I. S. Severina, *Biokhimiya*, **52**, 956 (1987).
23. D. L. Garbers and F. Murad, *Adv. Cycl. Nucl. Res.*, **10**, 57 (1979).
24. M. M. Bradford, *Analyt. Biochem.*, **72**, 248 (1976).