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# Competitive Inhibition of Swine Kidney Copper Amine Oxidase by Drugs: Amiloride, Clonidine, and Gabexate Mesylate

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**Competitive inhibition of swine kidney copper amine oxidase by diuretic, antihypertensive, and anticoagulant drugs, amiloride, clonidine, and gabexate mesylate, respectively, is reported. The affinity of these compounds for swine kidney copper amine oxidase is similar to that observed for inhibitor binding to nitric oxide synthase and trypsin-like serine proteinases. This finding suggests that amiloride, clonidine, and gabexate mesylate should be administrated under careful control, since enzyme cross-inhibition may occur also *in vivo*.** © 1997 Academic Press

***Key Words:*** **swine kidney copper amine oxidase; amiloride; clonidine; gabexate mesylate; competitive enzyme inhibition.**

Copper amine oxidases (E.C. 1.4.3.6) have been identified in bacteria, in yeasts and filamentous fungi, plants and animals. These enzymes are homodimers of 70-95 kDa subunits, each containing a single copper ion and a covalently bound cofactor formed by the post-translational modification of the catalytic tyrosyl residue to 2,4,5-trihydroxyphenylalanine quinone (TPQ). These enzymes catalyze the oxidative deamination of biogenic amines, including mono-, di- and poly-amines, neurotransmitters, histamine and xenobiotic amines with substrate preference depending upon the enzyme source. The enzyme reaction follows the general scheme:

$$E_{ox} + R\text{-}CH_2\text{-}NH_2 \rightarrow E_{red} + R\text{-}CHO \quad (1)$$

$$E_{red} + O_2 + H_2O \rightarrow E_{ox} + NH_3 + H_2O_2, \quad (2)$$

where $E_{ox}$ represents the ezyme-quinone, $R\text{-}CH_2\text{-}NH_2$ is the substrate, $E_{red}$ is the enzyme-aminoquinol, and R-CHO is the product aldehyde. Substrate amines interact directly with TPQ in the reductive half reaction forming a Shiff base complex (reaction 1). Proton abstraction of the substrate catalyzed by an invariant Asp residue leads to the release of product aldehyde and leaves the enzyme in the reduced aminoquinol form (reaction 1). The oxidative half process (reaction 2) leads to reoxidation of the aminoquinol cofactor with the release of ammonia and hydrogen peroxide [1,2].

In mammals, copper amine oxidase activity is highest in the kidney, small intestine and maternal placenta and may exert a protective role towards elevated levels of amines and histamine [3]. Copper amine oxidases catalyze the oxidation of agmatine, which has been recently recognized to be an important bioactive molecule, being identified as the endogenous ligand for imidazoline receptors [4]. Agmatine is a good substrate for swine kidney copper amine oxidase, its $k_{cat}/K_m$ ratio being similar to that for putrescine [4]. Swine kidney exibits the highest agmatine content among mammalian organs, raising the possibility that agmatine may be the true substrate for copper amine oxidase [5]. Moreover, agmatine, has been reported to be an endogenous inhibitor of nitric oxide synthase [6] and an inactivator of trypsin-like serine proteinases [7]. Therefore, drugs structurally related to agmatine may inhibit not only copper amine oxidases, but also nitric oxide synthase and trypsin-like serine proteinases, and thus represent a risk factor in several pathologies. In the present study, the inhibitory effect of diuretic, antihypertensive and anticoagulant drugs, notably amiloride, clonidine and gabexate mesylate, respectively, on the swine kidney copper amine oxidase activity is reported.

## MATERIALS AND METHODS

Swine kidney copper amine oxidase was kindly provided by Prof. B. Mondovì (Department of Biochemical Sciences "A. Rossi Fanelli", University of Rome "La Sapienza", Rome, Italy).

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Abbreviation: TPQ, 2,4,5-trihydroxyphenylalanine quinone.

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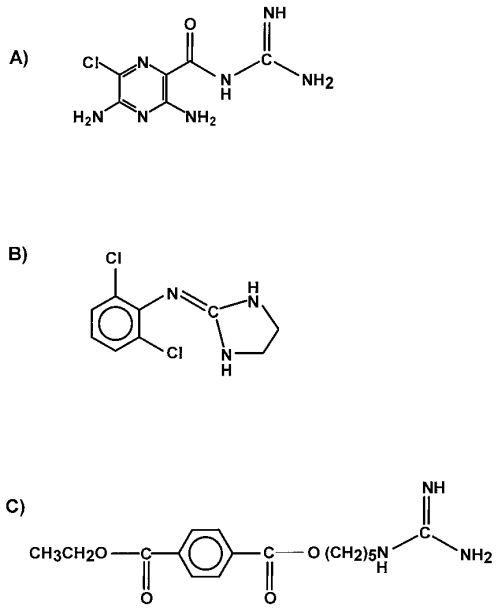


**FIG. 1.** Chemical structures of amiloride (A), clonidine (B), and gabexate mesylate (C). From [19].

Horseradish peroxidase, amiloride (see Fig. 1), aminoantipyrine, agmatine, clonidine (see Fig. 1), 3,5-dichloro-2-hydroxybenzenesulfonic and putrescine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Gabexate mesylate (see Fig. 1) was a kind gift of Lepetit S.p.A. (Milano, Italy). All chemicals were of analytical grade and were used without further purification.

Oxidation of agmatine and putrescine by swine kidney copper amine oxidase was investigated spectrophotometrically by following the formation of a pink adduct ($\epsilon_{515} = 2.6\times10^4\ M^{-1}\cdot cm^{-1}$), as a result of the oxidation of aminoantipyrine and 3,5-dichloro-2-hydroxybenzenesulfonic acid catalyzed by horseradish peroxidase [4], in the absence and presence of amiloride, clonidine and gabexate mesylate, at pH 7.0 ($1.0\times10^{-1}$ M phosphate buffer) and 25.0° C.

## RESULTS AND DISCUSSION

Amiloride, clonidine and gabexate mesylate inhibit competitively agmatine and putrescine oxidation catalyzed by swine kidney copper amine oxidase (see Fig. 2). As expected, values of $K_i$ were independent of the substrate, agmatine or putrescine. The affinity of amiloride for swine kidney copper amine oxidase is similar to that observed for inhibitor binding to human kidney copper amine oxidase [8]. Amiloride inhibits also nitric oxide synthase [9] and human urinary plasminogen activator [10], and gabexate mesylate inactivates trypsin-like serine proteinases [11]. In parallel, agmatine inhibits competitively nitric oxide synthase and trypsin-like serine proteinases [6,7], 4′,6-diamidine 2-phenylindole inactivates competitively swine kidney copper amine oxidase and trypsin-like serine proteinases [12,13], and *p*-aminobenzamidine inhibits nitric oxide synthase and trypsin like serine proteinases [14].

Inspection of the three-dimensional structures of homologous copper amine oxidase from *E. coli* [15,16] and from *Pisum sativum* L. [17], and the competitive inhibition mode of amiloride, clonidine and gabexate mesylate of swine kidney copper amine oxidase (see Fig. 2) suggest a non-covalent electrostatic interaction(s) between the positively charged inhibitor and the enzyme negatively charged TPQ-Asp catalytic diad. However, allosteric effect(s) cannot be excluded. In this respect, it may be observed that amiloride, gabexate mesylate and related compounds bind to the primary specifity subsite of trypsin-like serine proteinases, the positively charged guanidino group of the inhibitor being salt-linked to the invariant negatively charged enzyme Asp189 residue [10,11].

As a whole, amiloride, clonidine and gabexate mesylate inhibit agmatine and putrescine oxidation catalyzed by copper amine oxidases, nitric oxide synthesis

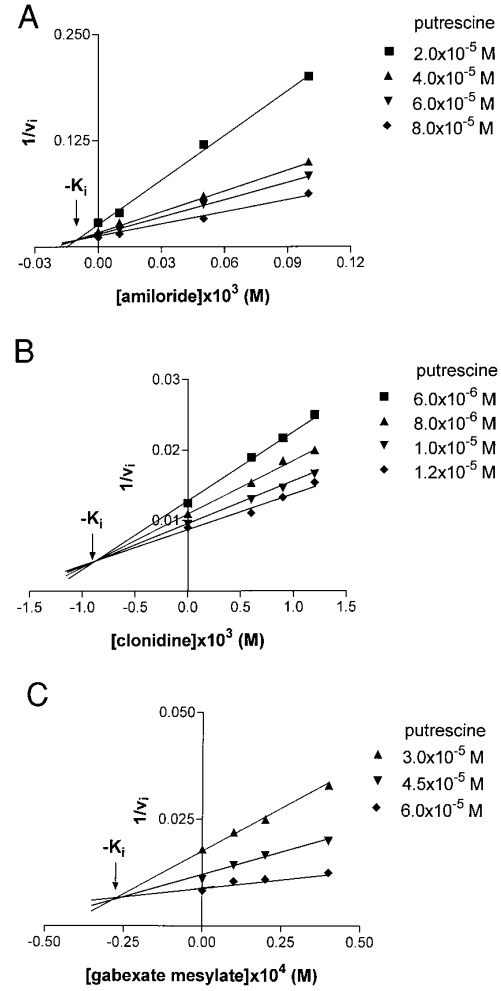


**FIG. 2.** Dixon plot for the inhibition of putrescine oxidation catalyzed by swine kidney copper amine oxidase by amiloride (A), clonidine (B), and gabexate mesylate (C); $v_i$, $\mu mol\cdot min^{-1}\cdot mg\ protein^{-1}$. Values of $K_i$ for amiloride, clonidine and gabexate mesylate binding to swine kidney copper amine oxidase are $1.0\times10^{-5}$ M, $9.0\times10^{-4}$ M and $2.7\times10^{-5}$ M, respectively, at pH 7.0 and 25.0°C.

induced by mouse brain nitric oxide synthase, and trypsin-like serine proteinase action [8-11]. Moreover, these compounds might also affect arginase, L-arginine-glycine transaminase, kyotorphine synthase and L-arginine decarboxylase, all using L-arginine as the substrate [18]. Therefore, amiloride, clonidine, gabexate mesylate and their analogues may affect (un)related function(s), modulated by enzymes acting on cationic substrates. Cross-inhibition of copper amine oxidases, nitric oxide synthase and trypsin-like serine proteinases may occur also *in vivo*. In particular, the decreased levels of nitric oxide, as a consequence of nitric oxide synthase inhibition, might induce some clinically-adverse amiloride reactions, such as an unexpected reduced antihypertensive effect.

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## REFERENCES

1. McIntire, W. S., and Hartmann, C. (1984) *in* Principles and Applications of Quinoproteins (Davison, V. L., Ed.), pp. 97–171, Dekker, New York.
2. Klinman, J. P. (1996) *Chem. Rev.* **96,** 2541–2561.
3. Sessa, A., and Perin, A. (1994) *Agents and Actions* **43,** 69–77.
4. Holt, A., and Baker, G. B. (1995) *Prog. Brain Res.* **106,** 187–197.
5. Stickle, D., Bohrer, A., Berger, R., Morrissey, J., Klahr, S., and Turk, J. (1996) *Anal. Biochem.* **238,** 129–136.
6. Galea, E., Regunathan, S., Eliopoulos, V., Feinstein, D. L., and Reis, D. J. (1996) *Biochem. J.* **316,** 247–249.
7. Barrett, A. J., and Salvesen, G. (Eds.) (1986) Proteinase Inhibitors, Elsevier, Amsterdam, New York, and Oxford.
8. Novotny, W. F., Chassande, O., Baker, M., Lazdunski, M., and Barbry, P. (1994) *J. Biol. Chem.* **269,** 9921–9925.
9. Ascenzi, P., Federico, R., Menegatti, E., and Venturini, G. (1997) *Biochem. Mol. Biol. Int.*, in press.
10. Vassalli, J.-D., and Belin, D. (1987) *FEBS Lett.* **214,** 187–191.
11. Menegatti, E., Bolognesi, M., Scalia, S., Bortolotti, F., Guarneri, M., and Ascenzi, P. (1986) *J. Pharm. Sci.* **75,** 1171–1174.
12. Cubría, C., Alvarez-Bujidos, M., Negro, A., Balaña-Fouce, R., and Ordóñez, D. (1993) *Comp. Biochem. Physiol.* **C105,** 251–254.
13. Casale, E., Collyer, C., Ascenzi, P., Balliano, G., Milla, P., Viola, F., Fasano, M., Menegatti, E., and Bolognesi, M. (1995) *Biophys. Chem.* **54,** 75–81.
14. Venturini, G., Menegatti, E., and Ascenzi, P. (1997) *Biochem. Biophys. Res. Commun.* **232,** 88–90.
15. Parsons, M. R., Convery, M. A., Wilmot, C. M., Yadav, K. D. S., Blakeley, V., Corner, A. S., Phillips, S. E. V., McPherson, M. J., and Knowles, P. F. (1995) *Structure* **3,** 1171–1184.
16. Wilmot, C. M., Murray, J. M., Alton, G., Parsons, M. R., Convery, M. A., Blakeley, V., Corner, A. S., Palcic, M. M., Knowles, P. F., McPherson, M. J., and Phillips, S. E. V. (1997) *Biochemistry* **36,** 1608–1620.
17. Kumar, V., Dooley, D. M., Freeman, H. C., Mitchell Guss, J. Harvey, I., McGuirl, M., Wilce, M. C. J., and Zubak, V. M. (1996) *Structure* **4,** 943–955.
18. Nakaki, T., and Kato, R. (1994) *Jpn. J. Pharmacol.* **66,** 167–171.
19. Budavari, S. (Ed.) (1996) The Merck Index, 12th Ed., Merck & Co., Whitehouse Station.