2-Bromoethylamine, a Suicide Inhibitor of Tissue-Bound Semicarbazide-Sensitive Amine Oxidase

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ABSTRACT—Various mammalian tissues contain plasma membrane-bound amine oxidase, termed semicarbazide-sensitive amine oxidase (SSAO). In the present study, 2-bromoethylamine has been studied with regard to inhibitory properties towards tissue-bound SSAO in rat lung. Without preincubation, 2-bromoethylamine was a competitive and reversible SSAO inhibitor with a Kᵢ value of 2.5 μM. After preincubation, it time-dependently and non-competitively inhibited SSAO activity, probably by forming the covalently-bound enzyme-inhibitor adduct. The data presented suggest that 2-bromoethylamine may act as a suicide inhibitor of SSAO.

Keywords: Semicarbazide-sensitive amine oxidase (SSAO), 2-Bromoethylamine (2-BEA), Suicide inhibitor

Semicarbazide-sensitive amine oxidase (SSAO) exists in plasma membranes of various tissues and blood plasma. SSAO is characterized by its relatively high activity towards the non-physiological amine benzylamine (BZ), which is also metabolized by monoamine oxidase (MAO). The affinity of SSAO towards BZ is considerably higher than that of MAO (1).

MAO activity is highly sensitive to inhibition by the suicide MAO inhibitors clorgyline, 1-deprenyl and pargyline (2). SSAO is resistant to these MAO inhibitors, but highly sensitive to carbonyl reagents such as semicarbazide. These differences in inhibitor selectivity between MAO and SSAO are frequently used to estimate their relative contribution in a particular reaction. As described above for BZ, the substrate specificities of both tissue-bound SSAO and MAO overlap to some extent (1, 3). Despite wide distribution of tissue-bound SSAO, its precise physiological role is still speculative. Roles of SSAO in pathophysiological processes such as blood vessel damage in diabetes mellitus (4) and in intra-cellular translocation of the GLUT4 glucose transporter in adipose cells (5) have, however, recently been reported.

To our knowledge, many SSAO inhibitors also inhibit MAO activity. Thus, a selective SSAO inhibitor(s) without any inhibitory effect on MAO may be a useful compound for studying the importance of SSAO. The enzyme-catalyzed conversion of a substrate to a highly reactive intermediate product leads to irreversible inhibition of the enzyme by forming a covalently bound enzyme-inhibitor adduct (6). Thus, the enzyme catalyzes its own inactivation. This type of inhibitor is called a suicide inhibitor or mechanism-based enzyme inactivator. Such a mechanism results in a high degree of inhibition and the lack of intrinsic reactivity might minimize the possibility of undesirable side-reaction(s).

We report here the suicide inhibition of rat lung-bound SSAO activity by 2-bromoethylamine (2-BEA). Findings obtained in this study were confirmed using guinea pig lung preparations and 2-BEA, and we previously reported them elsewhere (7), since enzymic properties of SSAO show considerable species-related variations (1).

Lungs of male Sprague-Dawley rats weighing about 250 g were used. The lungs were cut into small pieces and rinsed extensively in physiological saline to wash away any adhering blood as a source of contaminating plasma SSAO. The tissue was homogenized, then centrifuged at 600 × g for 10 min, and the resulting supernatant was used as the homogenate. All experiments complied with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

The radiochemical enzyme assay was performed at pH
7.8 and 37°C with 10 μM [14C]BZ as substrate. BZ oxidation by SSOA in rat tissues has $K_{inj}$ values of around 5 μM, which is much lower than for BZ metabolism by MAO (around 100 μM) (1), indicating that at this concentration, as reported earlier (8), almost all of the BZ oxidation was catalyzed by only SSOA. In addition, previous preliminary studies with 0.1 mM kynurenine used as the common substrate for three different amine oxidizing enzymes, the A- and B-form of MAO and SSOA showed that, even with 0.1 mM 2-BEA, no appreciable inhibition of both forms of MAO was detected in 0.1 mM semicarbazide pretreated preparations (7), showing that 2-BEA is a selective SSOA inhibitor, as so far tested. In inhibition studies, the enzyme preparations were preincubated at 37°C with 2-BEA for various time periods. Data are expressed as means, and if necessary, ± S.D.

Without preincubation, 2-BEA concentration-dependently inhibited SSOA activity with the IC₅₀ value of 15 μM. When the enzyme preparation was preincubated with $10^{-3}$ – $10^{-9}$ M 2-BEA for various time periods, the SSOA inhibitory activity of this compound became time-dependently enhanced. The degrees of SSOA inhibition increased with increasing time periods, and the inhibition was completed within 2 h; thus the SSOA inhibition curve shifted to lower 2-BEA concentrations with an IC₅₀ value of 0.28 μM.

Without preincubation, the mode of inhibition of SSOA by 2-BEA (Fig. 1, left) was competitive with the $K_{i}$ value of 2.5 μM. After a 2-h preincubation, its mode of inhibition changed to be non-competitive (Fig. 1, right). The time-dependency of an inhibitory process has often been used to show that it is irreversible. Dialysis experiments using 2-BEA-pretreated homogenates for 2 h resulted in no recovery of the activity even after overnight dialysis, indicating that the inhibition is irreversible. The different modes of inhibition with and without preincubation agree well with findings for covalently-bound MAO inhibition by suicide MAO inhibitors (9). The possibility that 2-BEA is a suicide SSOA inhibitor was also supported by the following finding: The rate of inhibition of SSOA activity by 2-BEA followed pseudo-first order kinetics when the residual SSOA activity was plotted as a linear regression of a log₁₀ scale of % remaining activities against preincubation times (Fig. 2, left). The classification as a suicide inhibitor indicates that the inhibitor (I) and the enzyme (E) combine to form a reversible complex (E.I.) as an intermediate before the covalently-bound enzyme-inhibitor adduct (E−I) is produced (7, 10), as shown below.

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E + I \rightleftharpoons E.I. \rightleftharpoons E-I
\]

Where $K_{D}$ is the apparent dissociation constant ($k_{-1}/k_{+1}$) of the reversible E.I. complex. Inhibition following this mechanism has been analyzed by Kitz and Wilson (10). The apparent
first-order rate constant for activity loss by 2-BEA, $k'$, is given by equation (2) and it can be

$$k' = \frac{k_{\text{init}}}{K_i + 1}$$

obtained from a graph of $\log_{10}$ (% remaining activity) against preincubation time (Fig. 2, left). The $k_{\text{init}}$ value (1.0 min$^{-1}$) and $K_i$ value (17.0 ± 1.1 μM) can be determined from a plot of $1/k'$ against $1/(2$-BEA) (Fig. 2, right). The $K_i$ value of 17 μM is similar to the $K_i$ value (about 3 μM). This fits well with the notion that if this compound acts as a substrate for SSAO, both values are similar. In the Kitz and Wilson analysis (10), the intersection of the plot of $1/k'$ vs. the 1/2-BEA concentration on the y-axis (Fig. 2, right) indicates that this haloamine is really a suicide SSAO inhibitor, but not an active-site directed affinity-labeling agent.

REFERENCES