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Inhibition of GABA shunt enzymes' activity by 4-hydroxybenzaldehyde derivatives

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Abstract—4-Hydroxybenzaldehyde (HBA) derivatives were examined as inhibitors for GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH). Investigation of structure–activity relation revealed that a carbonyl group or an amino group as well as a hydroxy group at the *para* position of the benzene ring are important for both enzymes' inhibition. HBA was shown to give competitive inhibition of GABA-T with respect to α-ketoglutarate and competitive inhibition of SSADH. 4-Hydroxybenzylamine (HBM) also showed the competitive inhibition on GABA-T with respect to GABA. In conclusion, the inhibitory effects of HBA and HBM on both enzymes could result from the similarity between both molecules and the two enzymes' substrates in structure, as well as the conjugative effect of the benzene ring. This suggested that the presence of the benzene ring may be accepted by the active site of both enzymes, HBA and HBM may be considered as lead compounds to design novel GABA-T inhibitors.

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system.[1] GABA is metabolized by the successive action of GABA transaminase (GABA-T), a pyridoxal 5′-phosphate (PLP)-dependent enzyme, and succinic semialdehyde dehydrogenase (SSADH), a NAD^+-dependent enzyme, to succinic acid, which is a substrate for the tricarboxylic acid (TCA) cycle.[2–5] Inhibition of the two enzymes in brain tissues increases the concentration of GABA and could have therapeutic applications in neurological disorders including epilepsy, Parkinson's disease, Huntington's chorea, and Alzheimer's disease. Recently, it has been found that an increase in GABA also blocks the effects of drug addiction.[6] In fact, the administration of GABA-T and SSADH inhibitors, such as valproate[7] or vigabatrin,[8,9] elevates GABA concentration, resulting in regional inhibitory effects.

4-Hydroxybenzaldehyde (HBA) is a major active constituent of Gastrodiae Rhizoma (GR), which is a very important Chinese herbal medicine used for the medical treatment of headaches, migraine, dizziness, epilepsy, rheumatism, neuralgia, paralysis, and other neuralgic and nervous disorders.[10] Previous studies showed that HBA potently inhibits GABA-T and SSADH activity,[11,12] and there was the report suggesting that the aldehyde group and the hydroxy at C-4 are necessary for the action.[13] However, the inhibition has been not still rationalized. Moreover, no further studies on the inhibitory pattern of HBA for both enzymes' activity have been reported, and so far the structure–activity relationship of SSADH has not been investigated. In this work, we attempted to elucidate the inhibition by investigating the structure–activity correlation of more HBA derivatives and the inhibitory pattern.

GABA-T and SSADH activity upon the introduction of varying concentrations of these compounds was determined using a modified assay of Qiu et al.[14] and a modified assay of Chambliss and Gibson,[15] respectively. The procedure and the assay solution accord to the above reports,[14,15] but the amount of NADH generated was measured by a HITACHI FL-4500 Fluorescence Spectrophotometer (excitation 355 nm, emission 459 nm). Investigation with 15 derivatives of HBA showed a structure–activity correlation (Table 1) differing from the previous study.[13] Among them, syntheses of compounds **3**, **6**, **7**, **9**, and **10** were achieved by the general methods in this laboratory and their structures were identified by NMR and MS. The IC_{50} values of compounds **1**–**6** on both enzymes' inhibition significantly

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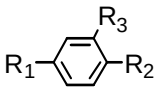
Table 1. Structures of compounds in this work and their IC_{50} values for the two enzymes

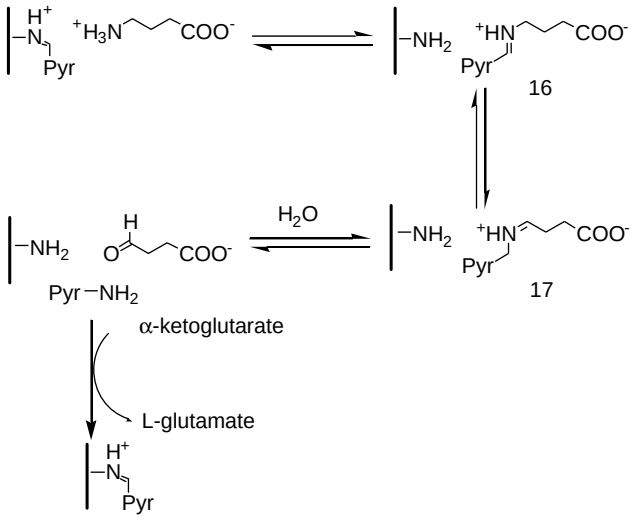


Compound	R_1	R_2	R_3	IC_{50} (μM)[a]	
				GABA-T	SSADH
1	CHO	OH	H	16.5	24.7
2	CHO	OH	OCH_3	17.1	15.6
3	CHO	OH	NO_2	15.5	33.1
4	$COCH_3$	OH	H	188	166
5	$COCH_2CH_3$	OH	H	176	293
6	$COCH{=}CH_2$	OH	H	5.48	0.35
7	CH_2NH_2	OH	H	15.4	>1000
8	CH_2OH	OH	H	>1000	>1000
9	$CH{=}NOH$	OH	H	>1000	>1000
10	$CH{=}NOH$	OH	OCH_3	>1000	>1000
11	CHO	OCH_3	H	>1000	>1000
12	CH_2OH	*O*-β-D-Glc	H	>1000	>1000
13	CHO	*O*-β-D-Allo	H	>1000	>1000
14	COOH	OH	H	>1000	>1000
15	COOH	NH_2	H	>1000	>1000

[a] $n = 3$.

differ from those of the others, demonstrating the importance of the carbonyl group and the hydroxy group at the benzene ring on the inhibition. The inhibitory effects of compounds **4** and **5** are significantly lower than that of HBA, suggesting that introduction of the alkyl group may reduce their potency. Surprisingly, however, replacement of a vinyl group caused a remarkable increase in the inhibition, which is worth investigating further.

The type of inhibition by HBA was studied further. In the reaction catalyzed by GABA-T, GABA is degraded to succinic semialdehyde and the PLP is converted to pyridoxamine 5′-phosphate (PMP), which is restored to PLP by transamination with α-ketoglutarate, generating the excitatory neurotransmitter L-glutamate (Scheme 1).[16] Therefore, competitive experiments with respect to both substrates were carried out. HBA was found to demonstrate a competitive action with respect to α-ketoglutarate (Fig. 1), but non-competitive inhibition by HBA with respect to GABA was observed (not shown). Furthermore, HBA inhibited SSADH activity in a competitive manner (Fig. 2).



Scheme 1. Catalytic mechanism for GABA-T.

The competitive action implied that HBA could be similar to SSA or α-ketoglutarate in structure. In fact, all the three molecules possess an aldehyde group and an acidic group such as a carboxyl group or a phenolic hydroxy group, together with an appropriate distance between both functional groups. Considering that substitution of the aldehyde group of SSA by an amino group should be the same as that of HBA, we speculate that 4-hydroxybenzylamine (HBM, **7**) may inhibit GABA-T activity. Our experiment indeed validated the naive hypothesis (Fig. 3). Interestingly, however, at concentrations of HBM beyond 80 μM, even at 300 μM, 10% of GABA-T activity was still observed. Competition experiments with increasing doses of GABA showed that HBM inhibited SSADH activity in a competitive manner with respect to GABA (Fig. 4).

In light of the above results, the possible mechanism of the inhibition of GABA-T by both molecules was proposed. HBA and HBM can bind to pyridoxamine 5′-phosphate (PMP) and pyridoxal 5′-phosphate (PLP) to form intermediates **19** and **18**, because SSA and GABA

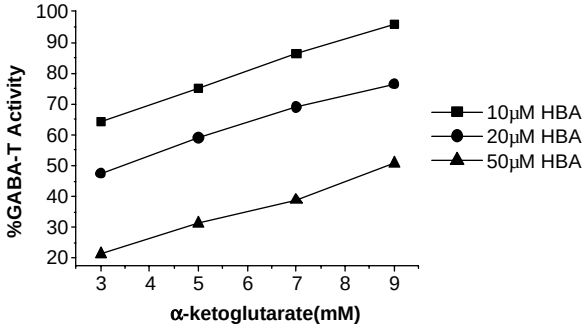


Figure 1. Effect of increasing doses of α-ketoglutarate on GABA-T activity in the different concentrations of HBA.

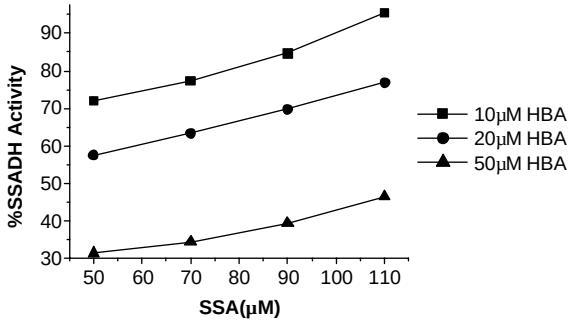


Figure 2. Effect of increasing doses of SSA on SSADH activity in the different concentrations of HBA.

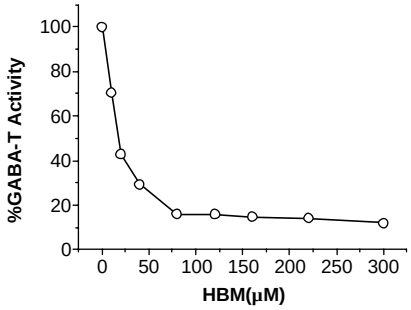


Figure 3. Inhibitory effect of HBM on GABA-T activity.

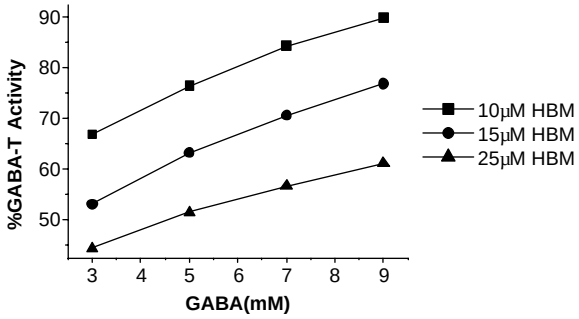
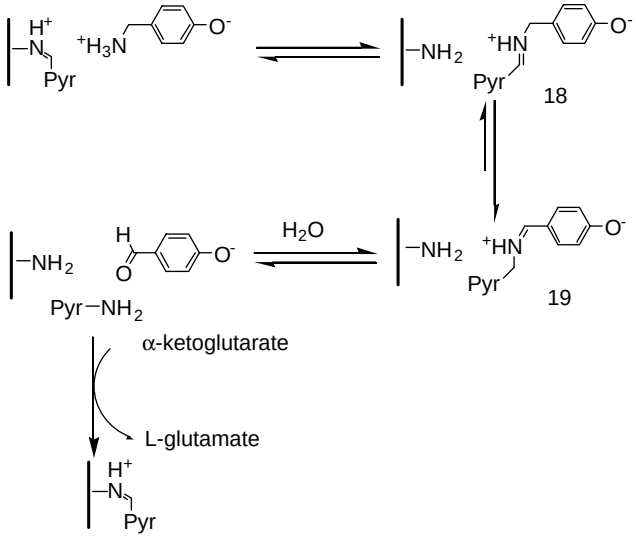


Figure 4. Inhibition of GABA-T activity by HBM is of a competitive manner with respect to GABA.

are similar to HBA and HBM in structure, respectively (Scheme 2). Intermediate **19** is more stable than **17** because of the conjugative effect of the benzene ring, resulting in competitive inhibition. However, there are presently no sufficient evidences indicating that intermediate **18** is more stable than **19**, thus the inhibition of GABA-T by HBM could be due to conversion from **18** to **19**. The inhibitory effect of HBA on SSADH can also be explained analogously. The aldehyde group of HBA or SSA as a hydrogen bonding acceptor can interact with a hydrogen bonding donor at the active site of SSADH, but the conjugative effect of the benzene ring stabilizes the HBA–enzyme complex.



Scheme 2. Possible mechanism of the inhibition of GABA-T by HBA and HBM.

Although further investigation is necessary to validate the mechanism, our data suggest that the presence of the benzene ring is accepted by the active site of both enzymes. So far, GABA-T inhibitors are almost GABA analogues without the benzene ring, hence this could result in a new class of GABA-T inhibitors. The structural similarity between HBM and GABA also implies that HBM could be a GABA receptor agonist and a GABA transport inhibitor.

It is also necessary to mention that the inhibitory effect on GABA-T activity decreased without a significance in the order: 3 > HBA > 2 (Table 1), according to their acidity order. As shown in Schemes 1 and 2, GABA and HBA as ion forms interact electrostatically with the positively charged residue of GABA-T, thus increasing acidity of GABA-T inhibitors may potentiate the inhibition. On the other hand, the inhibition of SSADH showed the contrary order, implying that SSA and HBA derivatives as molecule forms could interact with the enzyme. Further investigation is necessary to interpret the preliminary results, but this suggestion is supported by the fact that succinic semialdehyde methyl ester[17] and 4-hydroxy-*trans*-2-nonenal[18] are the enzyme's substrates.

In summary, the importance of the carbonyl group or the amino group, as well as the hydroxy group at the *para* position of the benzene ring, and the competitive inhibition, supported the proposed possible mechanism. The inhibition of both enzymes by HBA and HBM could result from the similarity between both molecules and the two enzymes' substrates in structure, together with the conjugative effect of the benzene ring. The results demonstrated that the presence of the benzene ring may be accepted by the active site of both enzymes, suggesting future directions for the design of more potent GABA-T inhibitors.

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