THE EFFECT OF POLYAMINES ON THE ENZYMES OF THE 4-AMINOBUTYRIC ACID METABOLISM IN MOUSE BRAIN IN VITRO

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1. Introduction

4-Aminobutyric acid (GABA) functions in mammalian brain as an inhibitory neurotransmitter. The synthesis of GABA is catalysed by L-glutamate decarboxylase (EC 4.1.1.15, GAD) and it is degraded by the consecutive action of 4-aminobutyrate-2-oxoglutarate aminotransferase (EC 2.6.1.19, GABA-T) and succinic semialdehyde dehydrogenase (EC 1.2.1.16, SSA-DH). The activities of the GABA shunt enzymes listed above have been reported to be affected by monovalent and bivalent cations in vitro [1–6].

The aliphatic amines putrescine, spermidine and spermine, commonly referred to as polyamines, are present in relatively high concentrations in mammalian nervous tissue. The polyamines influence numerous biochemical functions, in which they have been regarded as acting as cations (reviewed [7–10]).

In vivo studies [11–13] suggest that there may be regulatory interrelations between polyamines and GABA in the mammalian nervous tissue. Here we investigate the effects of the 3 polyamines on the enzymes of the GABA shunt in vitro in order to obtain information on their possible roles in regulating the activities of these enzymes.

We report a significant inhibition of SSA-DH by spermidine and to a lesser degree by spermine. The reversible inhibition by spermidine was hyperbolic and non-competitive in respect of both of the substrates, succinic semialdehyde (SSA) and NAD. The app. K_i of spermidine was <0.5 mM. Spermidine and spermine also caused an indirect inhibition of GABA-T.

2. Materials and methods

SSA was synthetized from monosodium glutamate as in [2]. [U-^{14}C]GABA (4.6 mCi/mmol) was obtained from the Radiochemical Centre, Amersham and L-[U-^{14}C]glutamic acid (260 mCi/mmol) from the NEN Corp., Boston, MA. All other chemicals were from the usual commercial sources and of the highest purity available.

The brains of randomly selected adult male mice (30 ± 2 g) of the NMRI strain were used as the enzyme source. The enzyme activities were determined essentially as in [14] for GAD and in [15] for SSA-DH. The radiochemical method [16] as modified in [15] was used for the GABA-T assays. The reversibility of the SSA-DH inhibition by spermidine was tested by gel filtration using a ready-packed PD-10 desalting column (Pharmacia Fine Chemicals, Uppsala).

Since >5% of the initial concentration of SSA and NAD was converted in the assays of SSA-DH, the apparent kinetic constants were calculated using the integrated form of the Henri-Michaelis-Menten equation [17]. The amount of product formed (y) during the time t is plotted against t^{-1}. $\ln S_0/S_t$, where S_0 is the amount of substrate at zero time and S_t that after time t. The K_i values were calculated from a replot of the ratio of the app. K_m and V_{max} vs the concentration of inhibitor.

All constants were calculated from the plots using the method of least squares. Covariance analysis for the regression lines was used to test the significance of the differences in K_m and V_{max}. The significances of the values in table 1 vs the control values were tested using the Student's t-test.

3. Results

GAD activity was measured in a medium containing 20 mM pyridoxal 5′-phosphate, 1 mM dithiotreitol, 32.5 mM sodium L-glutamate, 0.1 μCi L-[U-^{14}C]glutamic acid, 75 mM sodium phosphate buffer (pH 6.8) and 0.5 ml enzyme preparation in 1 ml total vol. Incubation was for 60 min at 37°C. The effects of putrescine, spermidine and spermine on GAD activity were tested by including these in the reaction mixture as HCl salts in 5 mM final conc. Four duplicate determinations for the control experiments and each polyamine were performed. The control value for GAD activity was 41.7 ± 3.9 μmol CO_2 . g tissue (wet wt)$^{-1}$. 60 min^{-1}, and the assays containing the polyamines showed no significant differences from this (at least $P < 0.01$, data not included).

The effects of the polyamines on GABA-T and SSA-DH activity are shown in table 1. The results indicate that the activity of both of the enzymes is significantly decreased by spermidine and spermine.

The GABA-T assay is normally carried out in the presence of NAD, which makes it possible for the endogenous SSA-DH in the homogenate to convert the intermediate product, SSA, to succinic acid. As the inhibition of SSA-DH may be reflected in the activity of GABA-T through accumulating SSA [18], we have also determined the GABA-T activity in the absence of NAD. As NAD is excluded, the measured product will be SSA, which causes increasing product inhibition during the assay and makes the interpretation of the results difficult.

There were no marked differences in results when the effects of polyamines on GABA-T were assayed without NAD (table 1). Also the time curves of product formation in all the assays lacking NAD were essentially identical (data not shown). This suggests that the inhibition of GABA-T, when assayed in the presence of NAD, is caused by the accumulating SSA and not by direct action of spermidine and spermine on the enzyme.

Both the inhibition of SSA-DH and the indirect inhibition of GABA-T turned out to be reversible in respect of the 2 polyamines, when they were removed from the medium by gel filtration.

The kinetics of the strongest of the SSA-DH inhibitors present, spermidine, were studied by using varied SSA concentrations at constant excess of NAD and vice versa at different spermidine concentrations. The resulting integrated Henri-Michaelis-Menten plots are shown in fig.1. The slopes (i.e., K_m) of the lines for the inhibited reactions did not deviate significantly from that of the zero line in either case (at least $P < 0.01$), whereas the intersections with the ordinate (i.e., V_{max}) were significantly affected in both cases. This indicates that the inhibition of SSA-DH by spermidine is noncompetitive in respect of both of the substrates. It may also be seen from the plots that the inhibition of SSA-DH by spermidine obeys

Table 1
Effect of polyamines on the activity of mouse brain GABA-T and SSA-DH in vitro

Effector			
Control	100.0 ± 9.8 (8)	100.0 ± 6.4 (8)	100.0 ± 6.8 (8)
Putrescine	99.6 ± 11.1 (4)	95.4 ± 7.4 (4)	99.9 ± 8.2 (4)
Spermidine	94.7 ± 8.3 (8)	51.7 ± 9.9[a] (8)	47.4 ± 9.3[a] (8)
Spermine	102.0 ± 5.9 (4)	84.1 ± 4.4[a] (4)	78.8 ± 4.3[a] (4)

Relative activities ± SD of the no. determinations shown in parentheses. Significance vs control: [a]at least $P < 0.01$. The absolute control values for GABA-T without and with NAD were 21.4 ± 2.1 and 39.2 ± 2.5 μmol product . g tissue^{-1} . 60 min^{-1}, respectively and for SSA-DH 46.8 ± 3.2 μmol NADH . g tissue^{-1} . 60 min^{-1} . GABA-T activity was measured with 2.22 mg tissue (wet wt)/ml, the incubation time being 60 min at 22°C. The medium contained: 50 mM Tris–HCl (pH 8.5); 3 mM GABA; 0.1 μCi [U-^{14}C]GABA; 2 mM 2-oxoglutarate; 20 mM mercaptoethanol; 1 mM succinic acid; 0.9 mM NAD. Polyamine was 2 mM. The conditions for the SSA-DH assay were the same as for GABA-T, except that 0.4 mM-SSA was used instead of GABA and [U-^{14}C]GABA, 2-oxoglutarate and succinic acid were omitted

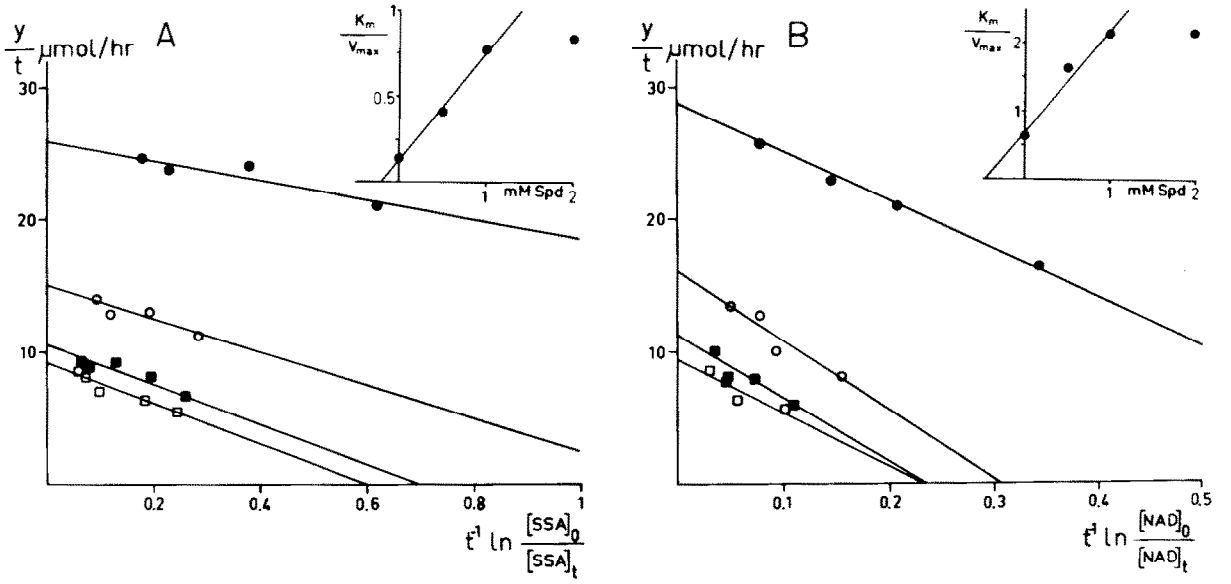

Fig.1. Integrated Henri-Michaelis-Menten curves to determine the effect of spermidine on the kinetic constants of SSA-DH. Incubation was carried out with 0.44 mg tissue (wet wt)/ml in 50 mM Tris–HCl (pH 8.5) at 22°C for 60 min in the presence of 8.2 mM 2-mercaptoethanol. (A) Inhibition by spermidine with respect to SSA. NAD was 0.25 mM. Spermidine: (•), no addition; (○), 0.5 mM; (■), 1 mM; (□), 2 mM. The points represent means of 3 separate determinations carried out in duplicate. A replot to determine the K_i of spermidine in respect of SSA is inserted. The point representing 2 mM spermidine concentration in the replot is excluded from the regression calculation, as it is clearly beyond the linear range of the inhibition. (B) Inhibition by spermidine in respect of NAD. SSA was 0.15 mM. The symbols are identical to those in (A). A similar replot for K_i determination is inserted.

hyperbolic saturation kinetics. The app. K_i values for spermidine, as calculated from the replots inserted in fig.1A,B are 0.20 mM and 0.47 mM, respectively.

The apparent kinetic constants for mouse brain SSA-DH as calculated from plot 1A are: V_{max} 52.3 µmol NADH . g tissue^{-1} . 60 min^{-1}; K_{SSA} 7.4 µM and from plot 1B: V_{max} 58.3 µmol NADH . g tissue^{-1} . 60 min^{-1}; K_{NAD} 36.9 µM.

4. Discussion

As the positively charged amino groups are the only functional components in the structure of the polyamines, it is obvious that these are responsible for the observed inhibition of SSA-DH. Since putrescine had no effect on the activity of SSA-DH, as seen from table 1, it seems that the specific arrangement of the amino groups in spermidine is necessary for the inhibitory action. The same arrangement of amino groups is included in the structure of spermine, but the additional propylamino group in its structure possibly causes the weakening of the inhibition as compared with spermidine. The fact that the inhibition of SSA-DH by spermidine was reversible, but independent of the concentration of the substrates, suggests that the site of action of the polyamine is different from the catalytically active site of the enzyme.

It has been postulated [18] that the metabolism of SSA could be regulated in vivo through the effects of monovalent and bivalent cations on the activity of SSA-DH. There is also evidence that changes in the activity of SSA-DH might be reflected in the cerebral concentration of GABA and thus affect the functioning of the nervous system [18]. The polyamines have certain advantages as compared with inorganic cations as possible regulators of SSA-DH, since their concentration can be regulated through metabolism in vivo. Active transport of polyamines within the nervous system [19,20] and into brain slices [21] has also been demonstrated.

We reported [12] that in vivo treatment of mice with a known GAD inhibitor, D,L-allylglycine, caused

a decrease in the brain GABA level and also affected the activities of enzymes of polyamine synthesis and the brain polyamine levels. Electrical stimulation, which has been reported to reduce GABA levels in cerebral cortex [22], also caused marked increase in the activities of the regulatory enzymes of polyamine synthesis [11]. It has also quite recently been reported that the increase of cerebral GABA level is reflected in the polyamine metabolism [13].

Spermidine and spermine in mouse brain are 0.33 and 0.30 mmol/kg wet wt, respectively, as determined from whole brain homogenate [12]. The spermine concentration is relatively stable between different areas of mammalian nervous system, while the amount of spermidine shows considerable regional variation [23–25], 0.34–1.4 mmol/kg having been reported for spermidine in different areas of the nervous system of the rat [25]. The K_i of spermidine for SSA-DH determined here, 0.35 mM (av. from the 2 plots), is seen to be within the range of physiological concentrations of this polyamine.

These results suggest that there is a specific arrangement of electrically negative sites in the SSA-DH molecule with which spermidine in particular is capable of interacting strongly, causing a significant reversible and noncompetitive inhibition of the enzyme. This finding might be of use when studying the structural features of the enzyme and when searching for specific inhibitors of SSA-DH. In spite of the known features mentioned above which suggest that spermidine in particular may be of physiological significance in regulating cerebral GABA metabolism, further research is required to establish its possible role in this connection.

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