THE INHIBITORY EFFECT OF SODIUM *n*-DIPROPYL ACETATE ON THE DEGRADATIVE ENZYMES OF THE GABA SHUNT

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1. Introduction

GABA may well be a major inhibitory transmitter in the mammalian CNS [1] and agents which inhibit its synthesising enzyme glutamate decarboxylase usually lower brain GABA levels and cause seizures [2]. Conversely, agents which raise brain GABA levels usually protect against convulsions. The latter compounds may well act by inhibiting the GABA degrading enzymes GABAT and SSAD. One such agent is sodium *n*-dipropyl acetate, the salt of an 8-carbon branched fatty acid, which is a recently introduced clinical anticonvulsant [3]. It has been shown to produce a 30–40% elevation of total brain GABA following a single intraperitoneal injection into mice, and the time of peak elevation of GABA corresponds to the time of maximum protection against seizures [4]. Following the extraction of brain GABAT and its assay in the presence of dPA, these authors have concluded that the rise in GABA concentration results from the inhibition of this enzyme. However, since the metabolic route of GABA degradation involves two enzymes, namely GABAT and SSAD, the possibility arises that more than one enzyme is inhibited by dPA. This paper reports an inhibition of SSAD by dPA which appears to be more potent than its action on GABAT.

Abbreviations: dPA: Sodium *n* - dipropylacetate; GABA: γ-amino butyric acid; GABAT: 4 amino-butyrate 2-oxoglutarate transminase (EC 2.6.1.19); SSA: Succinic semialdehyde: SSAD; Succinic semialdehyde dehydrogenase; OAAA: oxyaminoacetic acid.

2. Materials and methods

2.1. *SSAD and SSA preparation and assay*

SSAD was prepared from guinea pig kidney according to the method of Pitts et al. [5], and from rat brain by the method of Kammeraat and Velstra [6]. SSA was prepared by the method of Langheld [7] as modified by Bruce et al. [8]. A higher yield was obtained if the sodium hypochlorite and sodium glutamate were incubated for 60 min as opposed to the original 10 to 15 min. SSAD was assayed by the method of Kammaraat [6], the rate of reduction of NAD being followed fluorimetrically. The SSA preparations were assayed against standard NADH by employing SSAD.

2.2. *GABAT preparation and assay*

Partially purified GABAT was prepared from rat brain by Fowler's method [9], except that Triton X-100 was omitted from the initial homogenising medium as it interfered with the subsequent assay. Maximal activation of the enzyme was obtained instead by exposing the crude homogenate to ultrasound (15 kHz; 8 μ amplitude; stainless steel probe) for six bursts of 30 sec with cooling for 3 min between each exposure. The enzyme was assayed by the method of Balazs et al. [10]. This employs the transamination of SSA with glutamate to produce GABA, and the 2-oxoglutarate generated is reduced by glutamate dehydrogenase in the presence of excess ammonium ion. As the SSA preparation contained 2-oxoglutarate as an impurity, the reaction mixture was preincubated, in the absence of GABAT, and with excess NADPH (0.16 mM) for 10 min at room temperature to convert this 2-oxoglutarate to glutamate before starting the

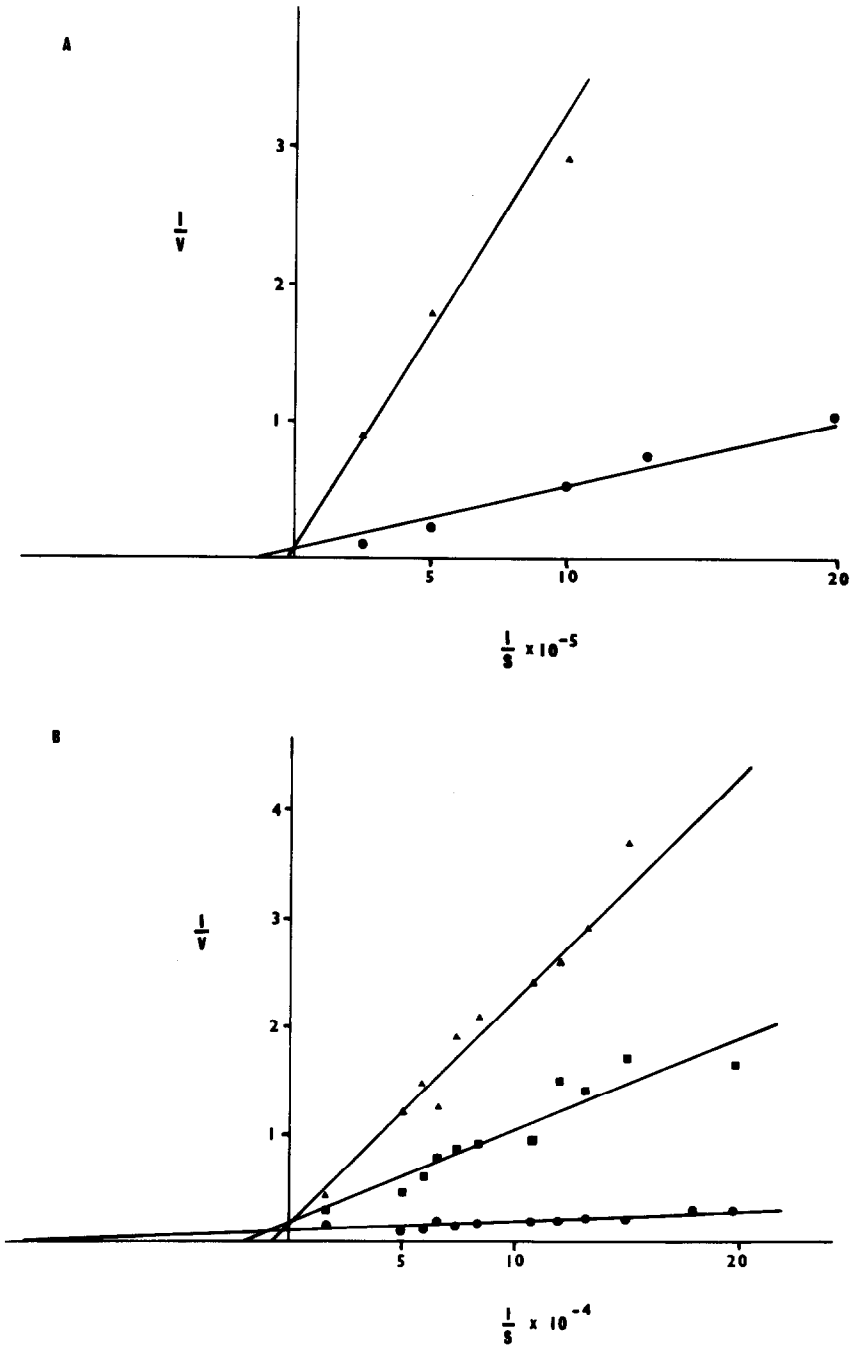

Fig.1. Regression lines for Lineweaver-Burk plots for SSAD: (●) SSA alone; (■) in the presence of 5 mM dPA; (▲) in the presence of 10 mM dPA. V is in arbitrary fluorometric units. Each point is the mean of between 6 and 10 experiments. A) Rat brain SSAD. SSA concentrations: 4×10^{-6} M–5×10^{-7} M. K_m (apparent) for SSA is 1×10^{-7} M. B) Guinea pig kidney SSAD. SSA concentrations: 6×10^{-5} M–5×10^{-6} M. K_m (apparent) for SSA 8.6×10^{-6} M.

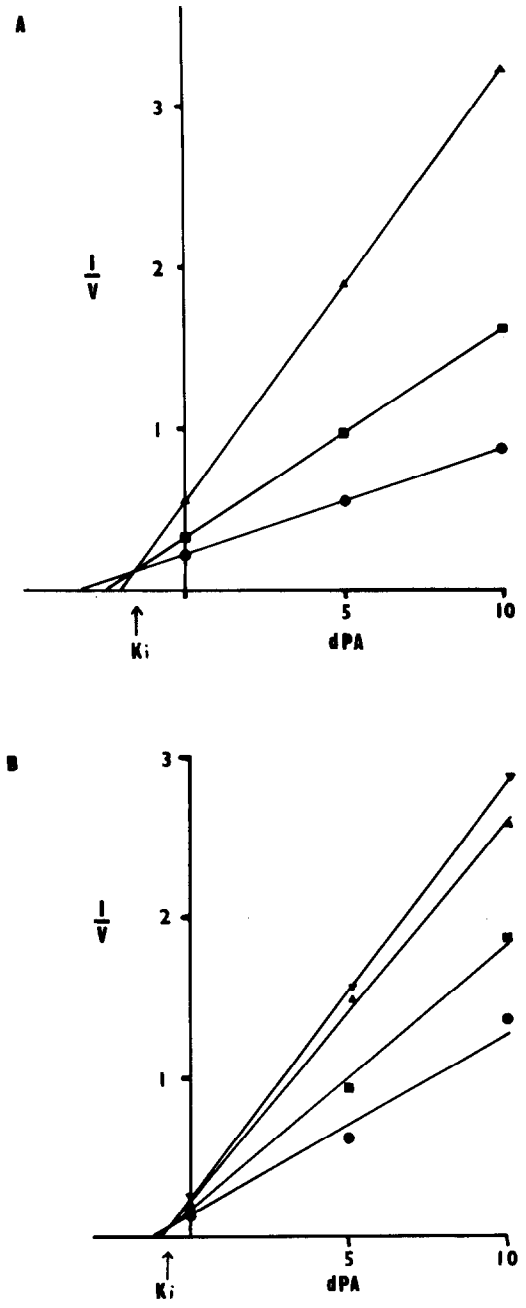

Fig.2. Determination of approximate K_i for inhibition of SSAD by dPA. V is in arbitrary fluorometric units, dPA concentrations in mM. A) Rat brain SSAD. SSA concentrations: (●) 4×10^{-6} M, (■) 2×10^{-6} M, (▲) 1×10^{-6} M. Ki is approximately 1.5×10^{-3} M. B) Guinea pig kidney SSAD. SSA concentrations: (●) 1.7×10^{-5} M, (■) 1.3×10^{-5} M, (▲) 0.8×10^{-5} M, (▼) 0.7×10^{-5} M. K_i is approximately 0.5×10^{-3} M.

assay by addition of GABAT. The GABAT preparation showed no SSAD activity and was totally inhibited by 1×10^{-6} M OAAA, a specific inhibitor of GABAT [9]. Glutamate dehydrogenase was not inhibited by dPA.

3. Results

3.1. *SSAD inhibition*

Conventional Lineweaver-Burk plots of $\frac{1}{V}$ against $\frac{1}{S}$ produced on apparent K_m of 1.0×10^{-7} M for brain SSAD, and 8.6×10^{-6} for the kidney enzyme (fig.1). Both were substantially and significantly inhibited by dPA (5 and 10 mM), with an apparent K_i of approximately 1.5×10^{-3} for brain and 0.5×10^{-3} M for kidney.

3.2. *GABAT activity*

The GABAT extracted from brain displayed an apparent K_m of 6×10^{-3} M. Although the data of fig.3 suggest an apparently competitive inhibition of GABAT by dPA with respect to SSA, and a non-competitive inhibition with respect to glutamate, analysis shows that there is no significant difference between the respective regression lines. It appears, therefore, that dPA is having little or no effect on the activity of this enzyme.

4. Discussion

The above results demonstrate that SSAD from rat brain and from guinea pig kidney is more potently inhibited by dPA than is GABAT from rat brain. It is therefore probable that SSAD provides the primary site of action of dPA in the GABA shunt pathway of brain. The effect of dPA on GABAT reported by Simler et al. [4] is probably due to an effect on SSAD; which, although initially present in their assay in excess, could have become rate-limiting following its inhibition by dPA. Maitre et al. [11], from the same laboratory, have examined the inhibition of GABAT by two analogues of dPA using a technique in which SSA is chemically measured, and not assayed enzymically employing SSAD. Although these analogues were effective inhibitors of GABAT, the data given

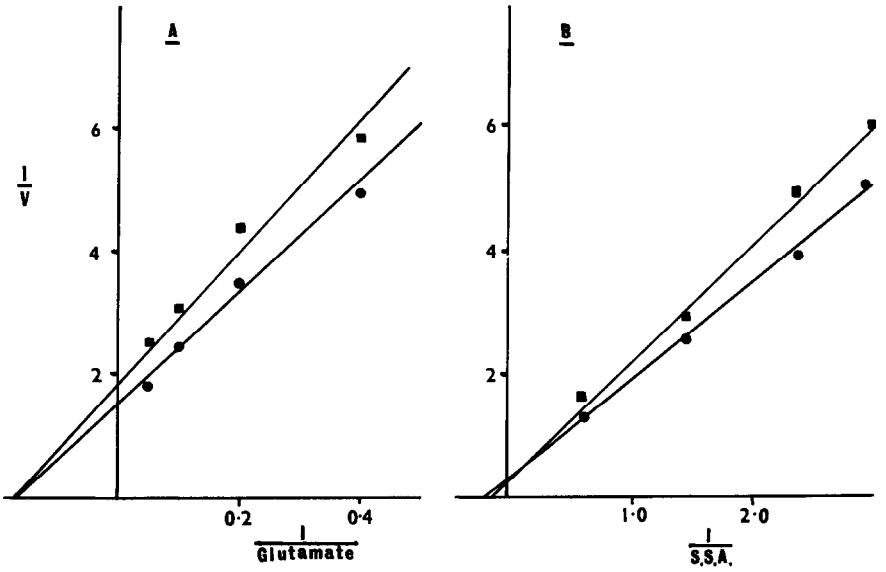

Fig. 3. Regression lines for Lineweaver-Burk plots for GABAT. Each point is the mean of at least six experiments. A) SSA. 1.7×10^{-3} M. (•) Glutamate 20×10^{-3} M–2.5×10^{-3} M, (▪) with 10 mM dPA. The difference between the two regression lines is not significant (P>0.05). B) Glutamate 10×10^{-3} M. (•) SSA 1.7×10^{-3} M–0.34×10^{-3} M, (▪) with 10 mM dPA. Difference between two regression lines is not significant. (P>0.1).

for dPA suggests a K_i value of 18×10^{-3}M.

The coincidence of peak GABA levels with the maximum anticonvulsant effect of dPA suggests a direct causal link between the two. However, raised intracellular GABA concentration will not necessarily lead to raised GABA levels at inhibitory receptors, and demonstrating a changed compartmentation of GABA in favour of an extracellular location is likely to be of more significance in the mode of action of dPA.

Acknowledgement

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