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SHORT COMMUNICATIONS

Effect of B-nitropropionic acid on rat brain acetylcholinesterase

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B-Nitropropionic acid is easily derived from B-propiolactone (now considered to be a carcinogenic substance [1]) by reaction with sodium nitrite [2]. It was isolated from higher plants and fungi [3]. It was found to be responsible for the toxic symptoms produced in dairy cattle [4]. Previous studies [5] indicated that B-propiolactone as well as B-nitropropionic acid have an inhibitory effect on rat brain monoamine oxidase (MAO) activity. The present study describes the *in vitro* effect of B-nitropropionic acid on rat brain acetylcholineesterase (AChE) activity.

Materials and methods

Partially purified (AChE) was prepared from albino rat brain according to the method of Harris *et al.* [6]. In this method the harvested brains (10 g) were homogenized in ice-cold 0.9% NaCl containing 0.01 M borate buffer (pH 8.8). The resulting homogenate was centrifuged twice at 600 *g* for 10 min to remove cellular debris. The supernatant was centrifuged at 10,000 *g* for 20 min and the resulting precipitate was further purified by repeated low and high centrifugation in the above mentioned buffer. The enzyme was assayed by the colorimetric method of Ellman *et al.* [7] as previously described [8]. In it the assay mixture contains 1.8 ml phosphate buffer (pH 8.0, 0.1 M) containing an amount of brain tissue equivalent to 0.08 units AChE, 70 μl dithio bisnitrobenzoic acid (DTNB) (0.01 M) and 13 μl acetylthiocholine iodide (AThChI) (21.67 mg/ml). Different concentrations of B-nitropropionic acid (9, 12, 18, 24, 30, 36, 48 and 72 μM) were added to the assay mixture to evaluate its inhibitory effect and I_{50} (the concentration of the inhibitor which inhibits 50% of the enzyme activity).

For determination of the type of inhibition and the enzyme–inhibitor dissociation constant (K_i), the substrate concentration was varied (80, 160, 240 and 320 μM) while the inhibitor (B-nitropropionic acid) was kept at a constant concentration for each experiment (6, 12 or 24 μM), and the mixture was immediately assayed.

Dialysis. The enzyme (1.8 ml containing 0.08 units AChE) with the inhibitor (48 μM) was dialyzed overnight against phosphate buffer (pH 8.0, 0.1 M) at 4° with occasional changes of the buffer. Controls of the enzyme without dialyzed and undialyzed inhibitor were also assayed.

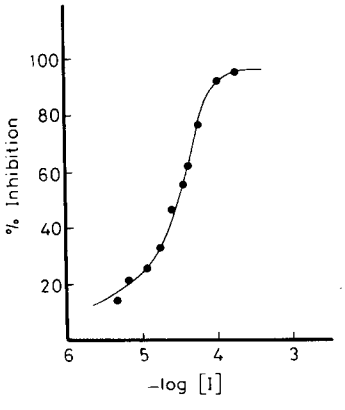

Fig. 1.

Results and discussion

Using a relatively low concentration of B-nitropropionic acid (9 μM) the inhibition of brain AChE in the presence of AThChI as substrate was 20%. By increasing the B-nitropropionic acid concentration from 12 to 72 μM the activity showed a further decrease and gave a sigmoid curve (Fig. 1). This behaviour of rat brain AChE inhibition is similar to that obtained in the case of rat brain MAO inhibition by the same inhibitor [5]. It was noticed that at the critical inhibitor concentration a further increase from 18 to 60 μM gave an abrupt increase in the inhibition of the enzyme. The curve also indicates that the enzyme lost 50% of its activity when the concentration of B-nitropropionic acid reached 27 μM.

By dialysis of the inhibited enzyme, it was found that the enzyme recovered about 90% of its original activity (Table 1) within 20 hr after dialysis suggesting that its inhibitory effect on brain AChE is reversible, which is similar to its effect on brain MAO [5].

With respect to the type of inhibition of AChE by B-nitropropionic acid, Fig. 2 shows that the double-reciprocal curves of $1/v$ (v is the velocity of the reaction) plotted against $1/[S]$ ($[S]$ is substrate concentration in moles) keeping B-nitropropionic acid at a constant concentration $[I]$ for each experiment (6, 12 or 24 μM) and changing the substrate (AThChI) concentration were in agreement with those mentioned by Dixon [9] for the competitive type of inhibition. The slope replots (inset of Fig. 2) show that B-nitropropionic acid was a linear competitive inhibitor {after the Cleland nomenclature (Mantle *et al.* [10])} of

Table 1. The effect of dialysis on the enzyme (AChE) activity before and after the addition of the inhibitor (B-nitropropionic acid)

Enzyme	Inhibitor (48 μM)	% inhibition
Without dialysis	Absent	
After dialysis	Absent	
Without dialysis	Present	76
After dialysis	Removed	10

Table 2. The enzyme–inhibitor dissociation constant (K_i) for brain AChE in the presence of B-nitropropionic acid (K_m is the Michaelis constant)

Constant	Value
K_i (calculated)*	18.8 ± 0.9 μM
K_i (slope replot)	18.0 μM
K_m	58.8 μM

* The mean value of K_i at different inhibitor concentrations (6, 12 and 24 μM).

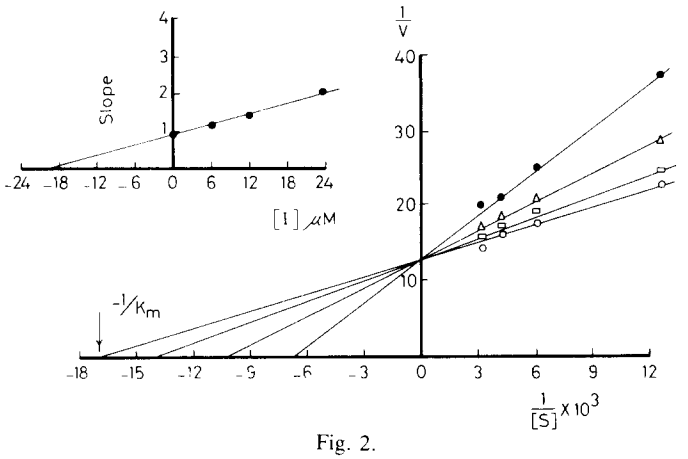

Fig. 2.

brain AChE. The calculated values of K_i and that found from the slope replot are in satisfactory agreement with each other. The Michaelis constant (K_m) is also given in Table 2.

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Cardiovascular and renal properties of potassium-sparing diuretics of the spirolactone group—I: effects of SC-14266/371 (potassium canrenoate) on renal transport Na^+,K^+-ATPase activity

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The maintenance of a high concentration of potassium and low concentrations of sodium inside the cell is the function of the sodium–potassium pump. Much evidence has accumulated suggesting that the transport of sodium and potassium ions across cell membranes is controlled by a specific ATPase system [1]. Evidence that Na^+,K^+-ATPase is the active component of the pump has been strong and overwhelming [2]. It is now widely held that inhibition of the Na^+,K^+-ATPase system by cardiac glycosides is responsible for the positive inotropic effect of these drugs [2].

Spirolactones are of vital importance in the management of patients with congestive heart failure (because of their potassium conservation action), and are used along with cardiac glycosides and other drugs such as the benzothiadiazine-type diuretics. Potassium canrenoate is one such spirolactone, which is water-soluble because of its open lactone ring (Fig. 1). It possesses mineralocorticoid antagonism similar to that of spironolactone in laboratory animals and man [3].

Potassium canrenoate also has a positive inotropic effect in man [4]. Like cardiac glycosides, this drug may be affecting the Na^+,K^+-ATPase activity. We tested this in a series of experiments designed to investigate the action of potassium canrenoate on renal microsomal Na^+,K^+-ATPase activity.

Albino rats of either sex weighing 200–250 g were used in all experiments. Microsomal fractions were prepared by centrifuging 10% kidney homogenates in 0.32 M sucrose/1 mM EDTA, as previously described [5]. ATPase activities were determined by measuring the release of inorganic phosphate from Tris–ATP (4 mmoles/l) in imidazole–HCl buffer (pH 7.4) as reported earlier [6]. All drug solutions were made up in imidazole–HCl buffer. The data presented are the mean of three to seven experiments and the criterion