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Effect of anticonvulsant drugs on the activity of acetyl CoA:arylamine *N*-acetyltransferase (EC 2.3.1.5) and hydroxyindole-*O*-methyltransferase (EC 2.1.1.4) from pineal gland

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There is evidence implicating the involvement of the pineal gland in seizure states [1, 2]. A number of reports attribute the anti-seizure activity of the pineal gland to the hormone melatonin [2–5]. In the light of these findings it is interesting that no reports have studied the effect of anticonvulsant drugs on the pineal gland and more specifically on the enzymes responsible for synthesis of melatonin.

The present study examined the influence of 12 anticonvulsant drugs on the activities of acetyl CoA:arylamine *N*-acetyltransferase (EC 2.3.1.5) (SNAT) and hydroxyindole-*O*-methyltransferase (EC 2.1.1.4) (HIOMT) *in vitro*. These two enzymes are responsible for production of melatonin from 5-hydroxytryptamine.

Methods. Rats of the Wistar strain (200–300 g) of both sexes were treated with isoprenaline HCl (25 mg/kg i.p.) 3 hr before being killed in order to induce SNAT levels in the pineal gland which was assayed as previously described [6]. This assay technique relied on the transfer of an acetyl group from [1-^{14}C]acetyl CoA [205 MBq/mmole (Amersham, U.K.)] to tryptamine HCl (Sigma, St. Louis, MO). Enzyme homogenates in 0.05 M phosphate buffer (pH 6.5) contained one substrate at a constant concn (3×10^{-3} M in the case of tryptamine and 1×10^{-4} M in the case of acetyl CoA) while the concn of the second substrate varied (2×10^{-6}–3×10^{-5} M in the case of acetyl CoA and 2×10^{-4}–3×10^{-3} M in the case of tryptamine). These homogenates were incubated at 20° for 1 hr in the presence and absence of the drugs used (Table 1). After incubation the reaction was terminated by the addition of 0.2 M borate buffer (pH 10) and [^{14}C]*N*-acetyltryptamine extracted into a toluene : isoamyl alcohol (97 : 3) mixture and quantitated.

Bovine pineal glands were collected shortly after death, homogenised in 0.15% KCl, centrifuged at 3000 *g* for 30 min to remove cell debris and the supernatant was lyophilised. This lyophilisate served as a source of HIOMT and prior to assay was dissolved in 0.05 M phosphate buffer (pH 7.9) (1 mg in 100 μl). This enzyme solution was assayed according to a previous report [7] in which *N*-acetylserotonin (Sigma) was *O*-methylated using *S*-adenosyl-L-[methyl-^{14}C]methionine [18.5 MBq/mmole (Amersham)] as a methyl donor. Enzyme incubates contained one substrate at a constant concn (4×10^{-4} M) while the concn of the second substrate varied (2×10^{-5}–3×10^{-4} M) and the reaction was monitored in the presence and absence of the various drugs (Table 1). Incubation was carried out at 42° for 1 hr, and extraction and quantitation were carried out as previously described.

The results were fitted to the Michaelis–Menten kinetic equation using computer-assisted iterative non-linear regression. The points generated for SNAT in the presence of sulthiame (STH) were reanalysed according to Dixon [8] in order to derive dissociation constants.

Results and discussion. None of the drugs tested affected HIOMT and only STH had an effect on SNAT (Fig. 1a and b). This effect was a mixed non-competitive inhibition with respect to tryptamine and the rate expression can be represented as follows [9]:

$$\frac{1}{V} = \frac{1}{V_s}\left(\frac{1 + \text{STH}}{K_{ii}}\right) + \frac{K_{ia}}{V_s}\left(\frac{1 + \text{STH}}{K_{is}}\right)\frac{1}{[\text{tryptamine}]} \quad (1)$$

where V is the velocity at a certain tryptamine concn [tryptamine], V_s is the maximum velocity. K_{ii} is the dissociation constant for STH from the E·tryptamine·STH complex, K_{ia} is the dissociation constant for the E·tryptamine complex and K_{is} is the dissociation constant for the E·STH complex.

Table 1. Anticonvulsant drugs used

Drug	Supplier	Concn used (μg/ml)
Acetazolamide	Sigma, U.S.A.	5–200
Beclamide	Rona, U.K.	5–100
Carbamazepine	Ciba-Geigy, R.S.A.	5–120
Clonazepam	Roche, R.S.A.	0.001–0.1
Diazepam	Roche, R.S.A.	0.2–8
Diphenylhydantoin Na	Parke-Davis, R.S.A.	5–200
Ethosuximide	Parke-Davis, R.S.A.	20–800
Pheneturide	Sapos SA, Switzerland	5–100
Phenobarbitone Na	May & Baker, R.S.A.	5–200
Primidone	ICI, R.S.A.	5–200
Sodium valproate	Reckitt & Colman, R.S.A.	20–1000
Sulthiame	Bayer, R.S.A.	2–20

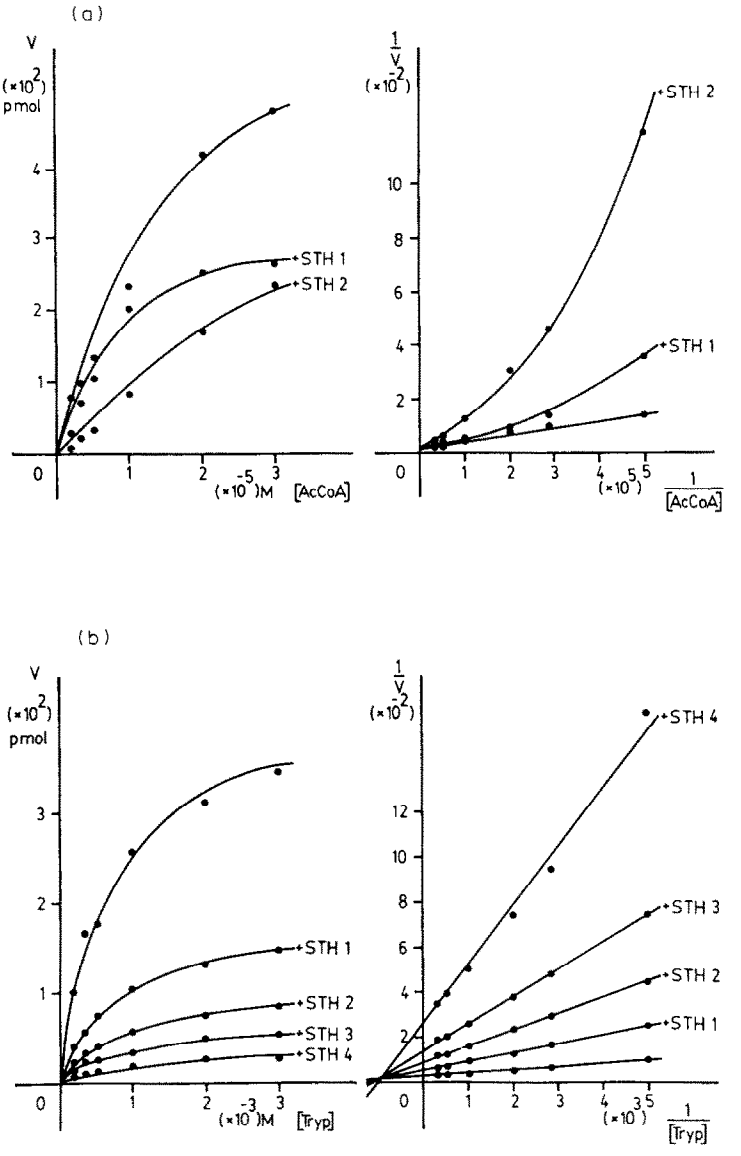

Fig. 1. Velocity v substrate concn for SNAT activity in the presence and absence of STH. Incubation was performed as described in the text. Double-reciprocal plots are shown to give an indication of the nature of the interaction. (a) STH effect on acetyl CoA, (b) STH effect on tryptamine (STH1 = 2 μg/ml, STH2 = 5 μg/ml, STH3 = 10 μg/ml, STH4 = 20 μg/ml). Velocity measured in pmoles *N*-acetyltryptamine formed per rat pineal gland per hour at 20°.

Table 2. Dissociation constants for the various complexes found between SNAT, tryptamine and STH calculated as described in the text

Dissociation constants (μM)
$K_{ia} = 547.44 \pm 17.64$
$K_{is} = 4.37 \pm 0.06$
$K_{ii} = 1.60 \pm 0.35$
$K_{im} = 200.93 \pm 6.47$

K_{ia} = dissociation constant for the E·tryptamine complex.
K_{is} = dissociation constant for the E·STH complex.
K_{ii} = dissociation constant for STH from the E·tryptamine·STH complex.
K_{im} = dissociation constant for tryptamine from the E·tryptamine·STH complex.

These dissociation constants are related to K_{im} (dissociation constant for tryptamine from the E·tryptamine·STH complex) in the following way [9]:

$$K_{im} = \frac{K_{ia} \cdot K_{ii}}{K_{is}}. \quad (2)$$

As the double-reciprocal plots are linear it may be assumed that the rapid-equilibrium rather than steady-state condition is met for the reaction.

Using the Dixon plot [8] in which the reciprocal of the velocity is plotted against the concn of the inhibitor at different fixed levels of tryptamine it is possible to obtain values for K_{ii} and K_{is} since the coordinates of the points of intersection of the lines are $-K_{is}$, $1/V_s(1 - K_{is}/K_{ii})$. From these values it is possible to calculate K_{ia} using equation (1) and hence K_{im} using equation (2) (Table 2).

When the u.v. spectra of STH, tryptamine and acetyl CoA were obtained in 0.05 M phosphate buffer (pH 6.5) (result not shown) it was interesting to note that there appeared to be an interaction between STH and tryptamine. This interaction may occur when tryptamine has bound to the enzyme and, although this is likely to interfere with catalysis, the major inhibitory effect of STH on SNAT can be assumed to be as a result of binding to the enzyme, such binding inhibiting the catalytic process in some way.

As the effect on catalysis is dependent on the STH concn but independent of the tryptamine concn it is likely that inhibition occurs at some stage after tryptamine has bound to the enzyme.

From the dissociation constants (Table 2) it is apparent that STH has a far greater affinity for the enzyme than tryptamine and also tends to bind better to the enzyme–tryptamine complex rather than to the enzyme alone. This favours the proposal that inhibition occurs at some stage after tryptamine binding to the enzyme.

Considering that the inhibition is largely dependent on the concn of acetyl CoA it is likely that STH exerts a competitive-type interaction with acetyl CoA for catalytic sites. These inhibition patterns are compatible with the mechanism being random Bi–Bi although preliminary studies indicate that the catalytic mechanism for SNAT is ordered Bi–Bi with tryptamine the first substrate to bind.*

This contradiction may be resolved as follows. The increasing non-linearity of the double-reciprocal plots with higher STH concn (acetyl CoA as substrate) suggests that the presence of the inhibitor in the catalytic process is introducing complex second-degree terms to the rate equation. As a result the plots may appear competitive whereas the interaction may not be. The interference exerted by STH probably results from the drug binding to the enzyme, such binding interfering with normal acceptance of acetyl CoA at the catalytic site, affecting acetyl transfer or product removal after catalysis.

To a certain extent the dissociation constants favour a competitive-type interaction with acetyl CoA in the framework of an ordered Bi–Bi catalytic mechanism. In the envisaged ordered Bi–Bi mechanism tryptamine binding would cause a change in the catalytic site which could then accept acetyl CoA; affinity of STH for SNAT follows a similar pattern in that affinity for the enzyme–tryptamine complex is greater than that for enzyme alone. Whether STH affects the catalytic site directly or indirectly is unknown.

Inhibition of SNAT by STH occurs in the normal therapeutic range of the drug (3–11 μg/ml) [10] and, considering that SNAT is the rate-limiting enzyme in the production of melatonin [11], it might be expected that a significant reduction in melatonin production should be observed in subjects taking STH. Considering the many effects postulated for melatonin [12] such an effect would be bound to cause some physiological changes. Whether this in fact occurs *in vivo* and what significance such an effect would have on anti-seizure therapy are unknown at this stage.

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School of Pharmaceutical Sciences
Rhodes University
Grahamstown
6140, Republic of South Africa

DOUGAL J. MORTON†

* Unpublished data.

† Address for correspondence: Department of Pharmacy, University of Zimbabwe, P.O. Box M P 167, Mount Pleasant, Harare, Zimbabwe.

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