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SHORT COMMUNICATIONS

Effect of *in vivo* anticonvulsant drugs on pineal gland indole metabolism in organ culture

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Several reports have implicated pineal gland involvement in seizure states [1, 2] with melatonin acting as the anticonvulsant principle [2–5]. To date it has been shown that sulthiame is a mixed non-competitive inhibitor of Acetyl Co A: Arylamine *N*-acetyltransferase (EC 2.3.1.5) (SNAT) *in vitro* [6] and that reduction of melatonin synthesis occurs after treatment with acetazolamide, carbamazepine, clonazepam, diazepam, diphenylhydantoin, primidone, sulthiame and valproate *in vitro* [7]. These *in vitro* studies show a reduction in melatonin synthesis following anticonvulsant treatment which would be expected to worsen seizures if melatonin is indeed a natural anticonvulsant substance. As *in vitro* and *in vivo* studies are not always comparable the effect of twelve anticonvulsant drugs *in vivo* on pineal gland indole metabolism was investigated in an attempt to clarify the relationship between anticonvulsants and pineal gland biochemistry and to ascertain whether melatonin could play a role in seizure control following anticonvulsant administration.

Treatment of rats

Male rats (180–220 g) kept under constant light conditions (04hr00–16hr00 light) were used. The rats were allowed at least two weeks in which to become acclimatized to the light cycle before being used. Rats were dosed twice daily with the various anticonvulsant drugs at 06hr00 and 15hr00 and were sacrificed at 15hr00 on the third day, pineal glands removed with a minimum of delay using sterile forceps and placed into culture medium. Drugs were dissolved or suspended in propylene glycol where insoluble in water and administered i.p. Both beclamide and pheneturide resisted solution and suspension and were given orally (see Table 1 for dosages).

Pineal glands were cultured in 50 μl BGJ.b. medium (Fitton-Jackson modification) containing 5-hydroxy [G-^{3}H] tryptamine creatinine sulphate (16 μM) and the anticonvulsant drugs at a concentration within the normal therapeutic range (see Table 1).

The glands were placed into culture shortly after sacrifice, tubes were gassed with 95% O_2/5% CO_2, sealed and incubated at 37° for 24 hr.

After incubation pineal glands were removed and 5 μl aliquots of the culture medium spotted onto pretreated thin layer chromatography (TLC) plates (Silica gel 60, Merck, Darmstadt, F.R.G.). The pretreating involved applying 5 μl of 95% ethanol containing 2.5 μg of each of the serotonin metabolites. These spots were dried prior to application of the culture media and served to localise the various metabolites after two dimensional development.

Control tubes containing medium and 5-hydroxy[G-^{3}H] tryptamine creatinine sulphate were treated in the same way except pineal glands were omitted and served to calculate background values for each metabolite.

TLC separation and quantitation

The spotted plates were dried and first developed in chloroform:methanol (9:1) followed by development in ethyl acetate at right angles to the first run. Before development and after each run the plates were dried by brief exposure to heat (65°). After development in ethyl acetate the plates were dried and sprayed with Van Urk's reagent (1 g 4-dimethylaminobenzaldehyde in 50 ml 25% hydrochloric acid and 50 ml ethanol) followed by heating at 95° for a short while. The coloured spots which resulted were scraped into scintillation cocktail and the radioactivity present was quantitated. Counting efficiency was determined using the external standards channel ratio and exceeded 20% in all cases.

Control incubation were treated in the same way, radioactivity resulting being subtracted from pineal values to obtain a true quantity for the metabolites produced.

Analysis of data

Counts per minutes (cpm) were converted into nanograms (ng) produced/pineal/24 hr with standard error of the mean (S.E.M) using computer assisted analysis. The specific activity of the metabolites varied due to removal of some of the original tritiated hydrogen atoms on serotonin. As a result a correction factor was built into the programs to enable accurate calculation of the quantities of metabolites produced. All determinations were repeated four times on separate occasions. The Student *t*-distribution was used to calculate probabilities.

Results and discussion

Parallel changes were observed with both hydroxyindole (Table 1) and methoxyindole (Table 1) production. Levels of *N*-acetylserotonin and melatonin were reduced by acetazolamide, beclamide, carbamazepine, clonazepam and sulthiame while levels of the other indoles were unaffected.

Reduction in production of *N*-acetylserotonin can be assumed to result from decreased SNAT levels as administration of these drugs *in vivo* has been shown to cause a decrease in SNAT activity [7, unpublished data]. The decreased melatonin production can be assumed to be due to reduced substrate availability as SNAT has been shown to be the rate limiting enzyme in production of melatonin [8–12].

An earlier report indicated that several of the anticonvulsants *in vitro* caused changes in pineal production of the other hydroxy- and methoxyindoles [7] which did not occur after *in vivo* administration. This difference can possibly be explained by compensatory mechanism/s resulting from repeated exposure to the drugs enabling the pineal to reinstate normal metabolic output. This report also found that diazepam, diphenylhydantoin, primidone and valproate *in vitro* led to a reduction in *N*-acetylserotonin and melatonin production [7] which, again, was not observed after *in vivo* administration. This may also be due to compensatory mechanism/s following repeated exposure leading to reinstatement of normal pineal output.

It is interesting that beclamide had no effect on indole metabolism *in vitro* [7] yet led to decreased *N*-acetylserotonin and melatonin production after *in vivo* administration (Table 1). This difference may be due to an active metabolite of beclamide formed *in vivo* or the effects of beclamide may be exerted at some extra-pineal site, presumably reducing sympathetic activity in the pineal. It

Table 1. Effect on *in vivo* anticonvulsant drugs on production of *N*-acetylserotonin (NAS), hydroxyindole acetic acid (HIAA), hydroxytryptophol (HTOL), melatonin (MTN), methoxyindole acetic acid (MIAA) and methoxytryptophol (MTOL) (ng produced/pineal/24 hr ± S.E.M.)

	Dosage	Concentration	NAS	HIAA	HTOL	MTN	MIAA	MTOL
No drug	—	—	5.3 ± 0.5	69 ± 11	12 ± 4	4.6 ± 0.4	5.2 ± 0.3	1.6 ± 0.3
Acetazolamide	100	10	3.0 ± 0.4†	61 ± 10	14 ± 5	2.2 ± 0.4†	5.8 ± 0.5	1.7 ± 0.5
Beclamide	250	5	3.3 ± 0.3†	63 ± 12	10 ± 3	2.1 ± 0.6†	5.5 ± 0.9	2.0 ± 0.4
Carbamazepine	25	6	3.6 ± 0.5*	57 ± 9	15 ± 6	2.8 ± 0.5*	5.4 ± 1.1	2.1 ± 0.5
Clonazepam	0.1	0.005	3.4 ± 0.4*	71 ± 13	14 ± 4	3.0 ± 0.5*	5.5 ± 0.9	1.7 ± 0.3
Diazepam	10	0.2	4.6 ± 0.8	61 ± 11	13 ± 4	4.2 ± 0.5	5.3 ± 0.7	1.8 ± 0.5
Diphenyhydantoin	100	10	4.4 ± 0.6	63 ± 12	10 ± 4	4.0 ± 0.6	5.6 ± 0.5	1.9 ± 0.4
Ethosuximide	25	40	5.2 ± 0.7	77 ± 13	14 ± 5	4.3 ± 0.6	5.3 ± 0.8	2.1 ± 0.6
Pheneturide	150	5	4.9 ± 0.6	68 ± 11	11 ± 5	4.5 ± 0.4	5.2 ± 0.7	1.5 ± 0.4
Phenobarbitone	12.5	10	4.0 ± 0.4	79 ± 14	16 ± 6	3.2 ± 0.5	5.5 ± 0.8	2.3 ± 0.8
Primidone	142	5	4.2 ± 0.4	81 ± 13	14 ± 5	3.4 ± 0.4	5.3 ± 0.6	2.2 ± 0.4
Sulthiame	50	5	3.9 ± 0.3*	78 ± 11	15 ± 4	3.4 ± 0.3†	5.6 ± 0.5	2.0 ± 0.5
Valproate	64	50	5.1 ± 0.5	71 ± 15	13 ± 3	4.3 ± 0.5	5.0 ± 0.7	2.3 ± 0.6

Dosage of drugs administered to rats (mg/kg) and concentration in the culture medium (μg/ml) are given.
* $P = 0.05$, † $P = 0.025$.

is also apparent, as the drugs reduced melatonin production, that this pineal hormone does not play a role in control of seizure states following anticonvulsant drug administration.

In conclusion, therefore, it is apparent that the pineal gland is capable of compensating to a certain extent for drug-induced changes in indole production and only five of the drugs tested caused significant reduction in melatonin output after chronic administration. It is possible that treatment with these drugs for longer periods may allow for compensatory mechanisms to return pineal indole output to normal and studies are at present underway to examine this possibility.

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Effect of 6-methylene-4-pregnene-3,20-dione treatment on hepatic bile acid sulfotransferase activity in male rats

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6-Methylene-4-pregnene-3,20-dione has been shown recently *in vitro* to be an irreversible inhibitor of prostatic Δ^4-3-ketosteroid-5α-reductase (5α-reductase) [1]. Detailed kinetic studies suggest that inhibition is dependent upon the presence of NADPH and involves two phases of interaction between the compound and the enzyme. In the first phase, 6-methylene-4-pregnene-3,20-dione binds reversibly to the enzyme; this binding can be shown to be competitive with the binding of the enzyme's natural substrate, testosterone. In the second interaction, the enzyme is irreversibly inhibited, presumably as the result of covalent binding of 6-methylene-4-pregnene-3,20-dione at the active site.

Treatment of male rats with 6-methylene-4-pregnene-3,20-dione results in marked regression of the weight of the ventral prostate and seminal vesicles after only 11 days [2]. These data suggest that 6-methylene-4-pregnene-3,20-dione also may be an effective antiandrogen drug *in vivo*.

Studies of bile acid metabolism have shown that the