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Impairment of cognitive function associated with hydroxyamylobarbitone accumulation in patients with renal insufficiency

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Summary

1. Sodium amylobarbitone was given by intravenous infusion to six patients with chronic renal insufficiency and to six healthy volunteer subjects. Serum concentrations of amylobarbitone and its major metabolite hydroxyamylobarbitone were measured by a gas chromatograph method.

2. The serum concentrations of amylobarbitone were consistently lower in the patient group than in the control group and the concentration half time was shorter ($0{\cdot}10>P>0{\cdot}05$); the 48 h urinary excretion of hydroxyamylobarbitone was reduced ($P<0{\cdot}001$) and the serum concentrations of hydroxyamylobarbitone were consistently raised.

3. When two patients were given 200 mg of sodium amylobarbitone daily over five consecutive days the serum concentration of hydroxyamylobarbitone rose steadily to a maximum of about 8 μg/ml. The serum concentrations in two healthy control subjects did not exceed 0·5 μg/ml.

4. Three parallel tests of cognitive function (Otis matched test forms A, B and C) were given to 16 control patients and to 12 amylobarbitone-treated patients. Significant impairment of performance was observed in test B ($P<0{\cdot}001$) at a time when amylobarbitone only could be detected in the patients' serum, and in test C ($P<0{\cdot}001$) when amylobarbitone concentrations were very low ($0{\cdot}52\pm0{\cdot}08$ μg/ml $\pm$ SEM) but hydroxyamylobarbitone concentrations were still high ($3{\cdot}30\pm1{\cdot}23$, μg/ml $\pm$ SEM).

5. There was a strong ($r=-0{\cdot}71$) and significant ($P<0{\cdot}01$) negative correlation between the performance in test C and the serum concentration of hydroxyamylobarbitone. It is concluded that hydroxyamylobarbitone has cerebral depressant effects in man.

Introduction

Pharmacological effects attributable to barbiturate metabolites have not previously been described in man. Waddell (1965) was unable to produce loss of consciousness in mice with very large doses of quinalbarbitone metabolites administered by injection. However, Irrgang (1965) found that the metabolite hydroxyamylobarbitone had about one-third the hypnotic potency of amylobarbitone in the mouse.

This study utilizes the accumulation of hydroxyamylobarbitone in patients with severely diminished renal function to assess the effect of this metabolite on cognitive function.

Methods

In the single dose studies sodium amylobarbitone (Sodium Amytal for injection: Eli Lilly & Co. Ltd.: 250 mg/70 kg) was administered as an intravenous infusion over 30 minutes. Venous blood samples were collected for the estimation of serum amylobarbitone and hydroxyamylobarbitone 12, 24, 36 and 48 h after the start of the infusion. The total urine output was collected in 12 h batches over the same period. The infusion was given to six healthy men (aged 26–41 years; weight 61–86 kg; urea clearance 40–80 ml/min) and to six men with chronic renal insufficiency (aged 23–46 years; weight 60–75 kg; urea clearance 1–6 ml/min).

In multiple dose studies one sodium amylobarbitone tablet (Sodium Amytal; Eli Lilly & Co. Ltd.: 200 mg) was given on each of five consecutive nights to two healthy men (urea clearance 40 and 80 ml/min) and to two male patients (urea clearance 2 and 3 ml/min). Venous blood was collected daily at noon.

In studies on cognitive function the tests (Otis Mental Examination: Higher Series: matched test forms A, B and C) were given to 28 out-patients with diminished renal function (urea clearance 2–12 ml/min) who are described in Tables 3 and 4. Most patients were still in full-time employment at the time of the studies and their levels of renal function were stable over the two week period. Sixteen patients constituted the control group and the remaining 12 patients received amylobarbitone.

Amylobarbitone-treated patients

Test A was administered at the first attendance when the patient was receiving no hypnotic drugs. A control blood sample was taken.

On the second attendance a few days later 200 mg of sodium amylobarbitone was given by intravenous infusion and test B was given one hour later. Blood was taken immediately before and after the test. The patient was then issued with four 200 mg tablets of sodium amylobarbitone and instructed to take one tablet on four consecutive nights following the night of the test day. Exactly seven days later test C was given and blood was taken.

The studies were explained in detail and each patient gave his consent, clearly understanding that no personal benefit was involved.

The control patients took Otis Tests A, B, C, according to the same schedule but no amylobarbitone was administered.

The tests

The Otis Self Administering Test of mental ability (Arthur S. Otis, Copyright 1950 of Harcourt, Brass & World Inc., U.S.A.) is a predominantly verbal multiple choice type, Group Intelligence Test with four parallel forms. It has been extensively standardized on American High School pupils and College students. A form takes 30 minutes to complete. Scores on the four forms are numerically equivalent but a mean re-test gain of 4 points (raw score) is predicted by the Test

Maker's Manual when a second test is administered a short time after the first test. A further gain in score is expected with a third test. Since the Test Manual Data apply to normal people and not necessarily to patients with renal insufficiency, a control group of patients was studied. The scores of the amylobarbitone-treated and the control groups were directly compared when assessing the effects of amylobarbitone and hydroxyamylobarbitone.

Determination of serum amylobarbitone and hydroxyamylobarbitone

Serum concentrations of amylobarbitone and hydroxyamylobarbitone were determined as described previously (Balasubramaniam, Mawer & Rodgers, 1969; Balasubramaniam, Lucas, Mawer & Simons, 1970; Mawer, Miller & Turnberg, 1972). An internal standard, quinalbarbitone in the case of amylobarbitone, and cyclobarbitone in the case of hydroxyamylobarbitone, was added to an aliquot of serum. The barbiturates were extracted into diethylether and the extracts were purified by chromatography on thin layer silica gel. The purified extracts were eluted and concentrated and then analysed by gas liquid chromatography using a flame ionization detector. The concentrations of amylobarbitone and hydroxyamylobarbitone were calculated from the peak area relative to the appropriate internal standard.

Samples of serum water for the determination of free hydroxyamylobarbitone were obtained by the ultrafiltration method of Walker (1967).

Gas liquid chromatography demonstrated that the amylobarbitone preparations contained a small amount of material with the same retention time as barbitone. This represented about 1% (w/w) of the total barbiturate.

Results

Disposition of amylobarbitone and hydroxyamylobarbitone

The results of single dose experiments are summarized in Table 1 and Figure 1. The serum concentrations of amylobarbitone in the uraemic patients were lower than the concentrations in the control subjects at all the times studied. The

TABLE 1. *(a) Serum concentrations of amylobarbitone and (b) urinary excretion of hydroxyamylobarbitone after the slow intravenous administration of sodium amylobarbitone (250 mg/70 kg) to healthy controls and to patients with severe chronic renal insufficiency*

Time (h)	Controls ($n=6$) Mean±S.E.	Patients ($n=6$) Mean±S.E.	t	P
(a) Serum amylobarbitone concentration (μg/ml)				
0	3·40±0·42	2·58±0·22	1·66	>0·10
12	2·49±0·24	1·64±0·19	1·96	>0·05
24	1·60±0·17	1·05±0·15	2·61	<0·05
36	1·08±0·09	0·68±0·13	2·45	<0·05
48	0·74±0·08	0·43±0·11	2·24	<0·05
Serum half time ($t_{1/2}$)	23·38±1·93	18·18±1·99	1·88	>0·05
(b) Urinary excretion of hydroxyamylobarbitone (% of dose)				
0–24	14·45±1·94	0·77±0·28	7·01	<0·001
0–48	31·33±4·60	2·91±0·77	6·12	<0·001

half time for the decay of serum amylobarbitone concentration between 12 and 48 h after intravenous administration was shorter in the patients than in the controls ($0{\cdot}10>P>0{\cdot}05$).

The urinary excretion of hydroxyamylobarbitone during the first 48 h after intravenous administration was markedly reduced in the uraemic patients ($P<0{\cdot}001$) (Table 1 and Fig. 1) and relatively high serum concentrations of hydroxyamylobarbitone were observed. Less than 20% of the serum hydroxy-

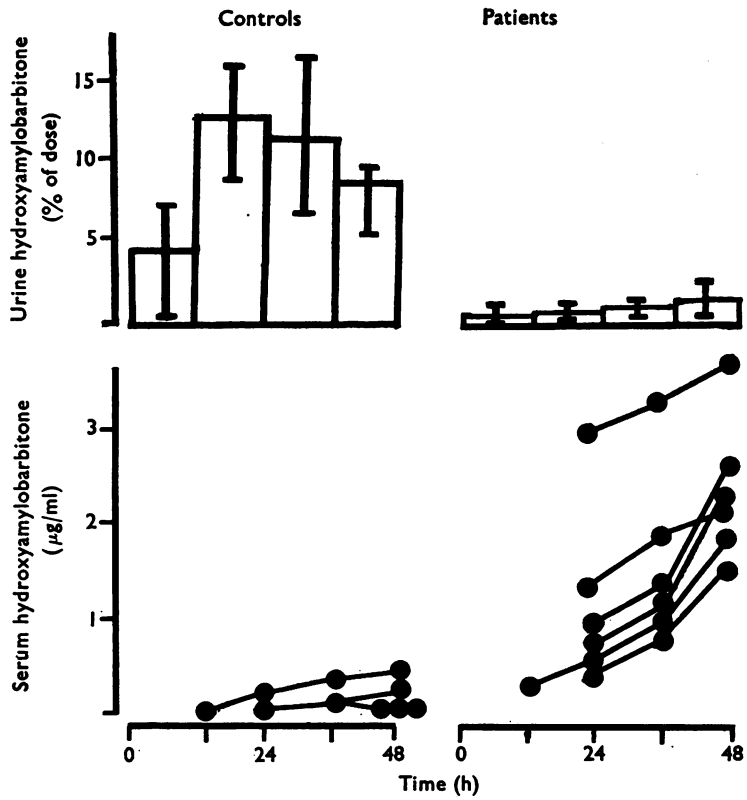

FIG. 1. Urinary excretion (mean±95% confidence limits) and serum concentrations of hydroxyamylobarbitone after the intravenous administration of sodium amylobarbitone (250 mg/70 kg) to 6 healthy subjects and 6 patients with renal insufficiency.

TABLE 2. *The renal clearance of free serum hydroxyamylobarbitone (C_{HO-A}) and endogenous urea (C_{urea}) in six uraemic patients*

C_{HO-A} (ml/min)	C_{urea} (ml/min)	C_{HO-A}/C_{urea}
0·6	1·0	0·60
3·2	2·0	1·60
1·1	2·1	0·52
2·4	2·5	0·96
2·9	2·6	1·12
4·0	6·0	0·60
Mean 2·37	2·70	0·90
S.E. 0·53	0·70	0·17

amylobarbitone was protein bound. The renal clearance of free serum hydroxyamylobarbitone in the six uraemic patients who had received intravenous amylobarbitone closely approximated to the renal clearance of endogenous urea (Table 2). The clearance of hydroxyamylobarbitone in the control subjects could not be calculated because of difficulty in the accurate measurement of the very low serum concentrations of hydroxyamylobarbitone; even when nightly doses of sodium amylobarbitone (200 mg) were given to control subjects (KB and GM) for as long as 21 days the mid-day serum concentrations of hydroxyamylobarbitone did not exceed 0·5 μg/ml.

When a hypnotic dose of sodium amylobarbitone (200 mg) was given to patients once nightly for 5 days the mid-day serum concentration of amylobarbitone approached a steady value but the serum concentration of hydroxyamylobarbitone rose daily during the period of administration (Fig. 2). Two days after the final dose of amylobarbitone the serum concentration of amylobarbitone had fallen to less than 1 μg/ml but the serum concentrations of hydroxyamylobarbitone remained relatively high (Fig. 2, Table 4).

Cognitive function studies and effects of hydroxyamylobarbitone accumulation

The performance of the control patients in the cognitive function tests is shown in Table 3. The second test (B) showed a significant increase in score over the first test (A) of $3{\cdot}3 \pm 1{\cdot}08$ (mean $\pm$ SE; $t=3{\cdot}06$, $P<0{\cdot}01$). Similarly the third test (C) showed a significant increase in score over the first test (A) of $7{\cdot}4 \pm 1{\cdot}36$

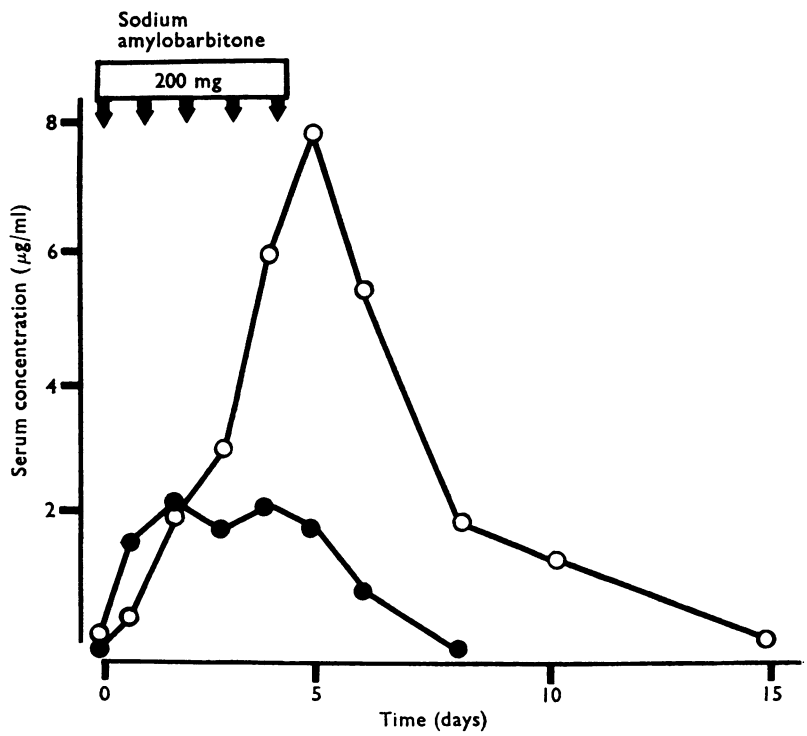

FIG. 2. The accumulation of serum hydroxyamylobarbitone in a patient with renal insufficiency (urea clearance 2 ml/min) given an oral dose of sodium amylobarbitone (200 mg) on each of 5 consecutive days. ●, Amylobarbitone; ○, hydroxyamylobarbitone.

(mean $\pm$ SE; $t=5{\cdot}46$, $P<0{\cdot}001$). There was no significant difference ($t=0{\cdot}65$, $P>0{\cdot}50$) between the retest gain (score B−score A) for the control group of patients and the retest gain of four for a large group of healthy subjects quoted in the test manual.

The group of patients with renal insufficiency who later received amylobarbitone included some more intelligent individuals than the control group (Test A score, Tables 3 and 4). The first dose of sodium amylobarbitone (200 mg) given by intravenous infusion produced a mean serum concentration of $3{\cdot}60 \pm 0{\cdot}74$ (μg/ml $\pm$ SE) before test B and $3{\cdot}25 \pm 0{\cdot}86$ (μg/ml $\pm$ SE) after test B. The normal retest gain (score B−score A) was reversed, the mean increment in score being $-6{\cdot}4 \pm 1{\cdot}24$ ($\pm$SE). The reduction of mean score in the group of patients that received amylobarbitone was significant ((B−A) control−(B−A) treated= $9{\cdot}7 \pm 1{\cdot}6$; $\pm$SE; $t=6{\cdot}60$, $P<0{\cdot}001$).

TABLE 3. *The performance of the control group of uraemic patients in three parallel forms (A, B and C) of the Otis Test of Mental Ability*

Patient	Age	Sex	Score Test A	Score Test B	Score Test C
1	36	M	23	26	32
2	27	M	30	40	36
3	44	M	44	48	59
4	50	F	7	10	—
5	53	F	38	39	44
6	45	M	38	40	41
7	32	M	46	45	57
8	24	F	53	54	61
9	47	M	40	48	54
10	35	M	45	56	57
11	26	F	26	27	31
12	63	M	14	18	—
13	50	F	13	19	17
14	29	M	23	23	28
15	53	M	41	37	38
Mean	—	—	32·0	35·3	42·6

TABLE 4. *The performance of the treated group of patients in three parallel forms of the Otis Test of Mental Ability (A) before, (B) during and (C) after the administration of five daily doses of sodium amylobarbitone* (200 *mg*)

			TEST A	TEST B		TEST C		
Patient	Age	Sex	Score	Score	Mean serum amylobarbitone concentration μg/ml	Score	Serum amylobarbitone concentration μg/ml	Serum hydroxyamylobarbitone concentration μg/ml
1	63	F	28	28	2·7	26	1·0	4·0
2	19	M	59	54	3·8	63	0·2[1]	1·8
3	47	F	46	33	4·3	41	0·2	5·2
4	44	F	53	47	2·8	56	0·2	4·4
5	52	M	62	60	3·0	60	0·4	4·3
6	56	M	23	15	5·0	20	0·7	3·3
7	43	M	13	7	3·4	13	0·6	4·0
8	53	M	20	10	3·1	18	0·8	3·0
9	39	M	22	17	2·8	22	0·7	2·8
10	31	F	69	63	2·4	72	0·7	2·2
11	35	F	51	37	3·4	55	0·2[1]	1·2
12	24	F	54	52	—	52	—	—[2]
Mean			41·7	35·2	3·3	41·5	0·5	3·3

[1] Patient omitted several doses of amylobarbitone. [2] Blood samples lost.

At the time of test C (Table 4) the mean serum concentration of hydroxyamylobarbitone was $3{\cdot}30 \pm 1{\cdot}23$ (μg/ml $\pm$ SE) and the mean serum concentration of residual amylobarbitone was $0{\cdot}52 \pm 0{\cdot}08$ (μg/ml $\pm$ SE). The normal retest gain (score C−score A) was not observed ; the mean increment in score was $-0{\cdot}17 \pm 0{\cdot}89$ ($\pm$ SE). The reduction of mean score in the group of patients with high serum concentrations of hydroxyamylobarbitone was significant ((C−A) control−(C−A) treated$=7{\cdot}42 \pm 1{\cdot}15$; $\pm$ SE ; $t=6{\cdot}43$, $P<0{\cdot}001$). There was a strong negative correlation between the performance in test C (score C−score A) and the serum concentration of hydroxyamylobarbitone ($r=-0{\cdot}71$, $n=11$, $P<0{\cdot}01$).

Discussion

In the single dose experiments there was no evidence of impairment of amylobarbitone metabolism in the patients with renal insufficiency. The serum concentrations of amylobarbitone were lower in the uraemic patients and the rate of decay of concentration was more rapid (Table 1). Similar observations have been made in uraemic patients given intravenous doses of phenytoin (Letteri, Mellk, Louis, Kutt, Durante & Glazko, 1971). It is not known whether the apparent increase in the rate of biotransformation is due to reduction of serum protein binding or to induction of microsomal drug oxidase. The phenomenon requires further investigation.

The renal clearance of hydroxyamylobarbitone appears to correlate with the renal clearance of urea (Table 2) and creatinine (Grove & Tosland, 1971). This accounts for the delayed elimination of hydroxyamylobarbitone by the patients with renal insufficiency (Fig. 1) and for the accumulation of hydroxyamylobarbitone in patients given a series of nightly doses of amylobarbitone (Table 4, Fig. 2).

The Otis Tests of Mental Ability were chosen as the instrument for cognitive function measurement because of the well standardized parallel forms. The mental examination was seen as a series of standardized problem solving tests rather than a determination of the patient's Intelligence Quotient.

The patients who had received amylobarbitone showed significant impairment of performance in test B when compared with the patient control group. At this time hydroxyamylobarbitone could not be detected in the serum.

The amylobarbitone-treated patient group also showed significant impairment of performance in test C. In view of the strong negative correlation between test C performance and the serum concentration of hydroxyamylobarbitone this effect is attributed to a depressant action of hydroxyamylobarbitone on cerebral function. The serum concentrations of amylobarbitone at that time are regarded as insignificant, though a contribution to the effect cannot be ruled out.

The effect cannot be attributed to accumulation of the trace contaminant barbitone for the cumulative dose was no greater than 10 mg.

It is concluded that hydroxyamylobarbitone the major metabolite of amylobarbitone possesses some of the cerebral depressant properties of the parent compound although the circumstances of these experiments did not permit the measurement of relative potency. The accumulation of hydroxyamylobarbitone studied, occurred on modest short-term dosage of amylobarbitone and may be considerably exceeded in clinical practice.

Dr. A. S. Curry generously gave a sample of hydroxyamylobarbitone and Mrs. E. M. Rodgers provided skilled technical assistance. Dr. K. Balasubramaniam was on postgraduate study leave from the University of Ceylon.

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