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BRAIN AND SERUM CALCIUM CONCENTRATIONS FOLLOWING ELECTROCONVULSIVE SHOCK OR BICUCULLINE-INDUCED CONVULSIONS IN RATS

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1 A single electroconvulsive shock (ECS; 125 V, 1 s, 50 Hz) given to rats anaesthetized with halothane produced little change in either serum or regional brain calcium concentrations, compared to rats anaesthetized with halothane, either 5 min or 24 h after the convulsion. Both anaesthetic and ECS-treated rats showed an elevated serum concentration 5 min after the ECS.

2 When 5 ECS were given spread out over 10 days there were no significant calcium concentration changes in either serum or brain except for an increase in the pons/medulla.

3 A single convulsion produced by bicuculline (0.375 mg/kg i.v.) resulted in a marked increase in the calcium concentration in serum, but not brain, 5 min later. Diazepam pretreatment (10 mg/kg i.v.) prevented both the convulsion and the serum calcium change.

4 Results are discussed in relation to clinical data on calcium changes following electroconvulsive therapy (ECT) and the enhanced monoamine-mediated behaviours which follow ECS administration to rats.

Introduction

Recently it has been found that after successful electroconvulsive therapy (ECT) there are consistent decreases in both serum and cerebrospinal fluid (CSF) calcium concentrations. These changes were not seen until after several ECT treatments had been given and appeared to be coincident with the antidepressant effect of the treatment. It was suggested that the changes in calcium metabolism might be associated with the mood improvement (Carman, Post, Goodwin & Bunney, 1977).

In experimental animals it has been shown that repeated electroconvulsive shock (ECS), when given in ways closely mimicking the clinical administration of ECT, produces enhanced monoamine-mediated behavioural responses (see review of Grahame-Smith, Green & Costain, 1978).

Since calcium is known to be intimately involved in the regulation of release of neurotransmitters including the monoamines (for example, Boullin, 1967; Stjärne, 1973 and see review of Rubin, 1970), we have investigated whether there are changes in brain and serum calcium following ECS administration to rats and whether such changes are associated with the altered behavioural responses seen in the animals. The effects of a bicuculline-induced convulsion on brain and serum calcium concentrations were also examined.

Methods

Animals

Adult male Sprague-Dawley derived rats (Charles River, Kent) weighing 150 to 200 g were used in all experiments. They were housed in groups in conditions of controlled temperature (21°C) and lighting (08 h 00 min to 20 h 00 min light period) and fed a diet of 41B pellets and tap water *ad libitum*.

ECS and drug administration

ECS was given through ear-clip electrodes to animals lightly anaesthetized with halothane. The ECS was normally 125 V, 50 Hz sinusoidal, for 1 s delivered from a Theratronics small animal electroplexy unit. Control animals received anaesthetic only.

Bicuculline (Sigma, Poole), diazepam (Roche) and 0.9% w/v NaCl solution (saline) were injected intravenously via a tail vein.

At various times after ECS or drug injection the rats were killed by decapitation, blood was collected into polycarbonate tubes, allowed to clot and serum taken for calcium analysis. The brain was removed and frozen at −20°C until dissection and analysis. The striatum, hypothalamus and pons/medulla were dissected by the method of Glowinski & Iversen (1966) and immediately weighed on a torsion balance to avoid weight changes due to dehydration.

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Calcium assays

Total serum calcium was measured by atomic absorption spectrophotometry on a Pye Unicam SP90 spectrophotometer after direct dilution of the serum with disodium edetate solution (Willis, 1960). Brain calcium was also measured by atomic absorption spectrophotometry using the method of Cardenas & Ross (1975).

Results were analysed statistically by Student's *t* test.

Results

Brain and serum calcium concentrations in untreated animals

The calcium concentrations in striatum, pons/medulla and hypothalamus were essentially the same as those reported by Cardenas & Ross (1975) and Ross (1976) (Table 1). Serum calcium values are also shown in Table 1.

Effect of a single ECS on brain and serum calcium concentrations

Five minutes after a single ECS there were no changes in either brain or serum calcium concentrations, other than an increase in the pons/medulla content, when compared to anaesthetic-treated controls (Table 1). This change was not seen, however, when the ECS results were compared to untreated controls (Table 1). Serum calcium concentrations in the ECS and anaesthetic group were elevated when compared to untreated rats (Table 1). Twenty-four hours after a single ECS, serum calcium remained elevated with respect to untreated rats, whereas levels in anaesthetic-treated controls had returned to the values of untreated animals. However, the difference between ECS- and anaesthetic-treated animals failed to reach statistical significance. The only significant change seen in the brain was an elevated calcium concentration in the hypothalamus although this increase was not apparent when the ECS values were compared to the untreated control values.

Effect of repeated ECS on serum and brain calcium concentrations

Enhanced monoamine-mediated behavioural responses are seen 24 h or more after the final ECS, when it has been given either once daily for 10 days or when 5 ECS have been given over a period of 10 days (Costain, Green & Grahame-Smith, 1979). We therefore examined the effects on serum and brain

Table 1 Effect of electroconvulsive shock (ECS) on serum and brain calcium concentrations

Treatment	*Time after last treatment of measurement*	*Serum calcium* (mg/100 ml)	*Brain calcium* (μg/g wet wt.) *Corpus striatum*	*Hypothalamus*	*Pons/medulla*
None	—	9.12 ± 0.23 (12)	50.8 ± 7.2 (10)	55.4 ± 10.5 (10)	52.4 ± 7.9 (6)
Anaesthetic × 1	5 min	9.74 ± 0.43† (8)	47.5 ± 1.6 (6)	58.7 ± 12.6 (7)	45.0 ± 3.6 (7)
ECS (1 s) × 1	5 min	9.56 ± 0.15† (7)	54.3 ± 12.6 (13)	57.0 ± 24.5 (13)	52.0 ± 6.1¶ (12)
ECS (3 s) × 1	5 min	9.23 ± 0.18§ (9)	42.8 ± 4.2**‡ (8)	48.4 ± 4.9† (9)	48.4 ± 1.4 (5)
Anaesthetic × 1	24 h	9.18 ± 0.51 (10)	58.2 ± 21.5 (10)	46.6 ± 8.5 (8)	54.6 ± 18.1 (9)
ECS (1 s) × 1	24 h	9.50 ± 0.59* (13)	61.1 ± 9.3* (7)	58.3 ± 11.9‡ (8)	59.9 ± 13.6 (9)
Anaesthetic × 5 (over 10 days)	24 h	9.56 ± 0.72 (11)	55.8 ± 10.7 (7)	58.5 ± 8.6 (7)	51.8 ± 7.7 (6)
ECS (1 s) × 5 (over 10 days)	24 h	9.40 ± 0.62 (15)	65.3 ± 12.7† (10)	52.0 ± 7.4 (10)	69.1 ± 15.9*‡ (11)

Values are the mean ±s.d. The number of samples are shown in parentheses. Number of ECS or anaesthetic administrations is given (×1 or ×5; the latter being given over 10 days: Mon, Wed, Fri, Mon, Wed). ECS conditions given in Methods. Results shown different from appropriate untreated rat values: * $P < 0.05$; ** $P < 0.02$; † $P < 0.01$. ECS values different from appropriate anaesthetic-treated control group: ‡ $P < 0.05$, ¶ $P < 0.02$; § $P < 0.01$.

calcium of 5 ECS administered over 10 days (Mon, Wed, Fri, Mon, Wed) with analysis 24 h after the final convulsion. Control rats were given 5 anaesthetic exposures using the same regime. The serum calcium concentration was not altered by ECS, nor was the calcium content of the hypothalamus. However there was a marked increase in the calcium content of the pons/medulla 24 h after the final ECS and an increase was also seen in the striatum although this was significant only when compared to untreated animals, not to anaesthetic-treated controls.

Effect of a 3 s ECS on serum and brain calcium concentrations

At no time following either a single or repeated ECS was a decrease seen in the serum calcium concentration, in contrast to the clinical data of Carman *et al.* (1977); nor was a decrease in brain calcium seen at any time after ECS administration. Since the period of the central seizure activity would presumably be longer in patients, the effect of a longer ECS was examined. Rats were given a single ECS of 125 V but for 3 s.

Five minutes after the convulsion, serum calcium decreased with respect to anaesthetic-treated controls but not when compared to untreated rats (Table 1). The calcium content of the striatum and hypothalamus was also decreased in ECS-treated rats when compared with anaesthetized controls, although this change was statistically significant only in the striatum when the values were compared to untreated animals.

Effect of a single bicuculline-induced convulsion on serum and brain calcium concentrations

To determine whether a chemically induced convulsion produced any significant changes in calcium, the effects of bicuculline were examined.

Rats were injected intravenously (via a tail vein) with either bicuculline (0.375 mg/kg) or saline (at an equivalent volume of 2.5 ml/kg). This dose of bicuculline produces a reproducible convulsion lasting 2 to 3 min (Nutt, Green & Grahame-Smith, unpublished observations). Five minutes after the injection, brain and serum calcium concentrations were measured. Intravenous saline produced a statistically significant lowering of serum calcium. However, bicuculline administration produced a large increase in calcium compared to either untreated or saline-injected rats (Table 2). Following intravenous saline the calcium concentration was lowered in striatum, hypothalamus and pons/medulla compared to untreated rats, although this change was statistically significant only in the pons/medulla. Furthermore, after bicuculline it was only in the pons/medulla that there was any marked change in calcium, compared to saline-treated rats, although the increase failed to reach statistical significance, possibly because of the large scatter of values seen after bicuculline administration (Table 2).

It seemed possible that the changes in serum calcium seen after a bicuculline-induced convulsion resulted from the prolonged increase in muscular activity, the period of convulsion being as long as 3 min. A group of rats was therefore pretreated with diaze-

Table 2 Effect of a bicuculline-induced convulsion on serum and brain calcium concentrations

Treatment	*Time after last treatment of measurement*	*Serum calcium* (mg/100 ml)	*Brain calcium* (μg/g wt wt.) *Corpus striatum*	*Hypothalamus*	*Pons/medulla*
None	—	9.12 ± 0.23 (12)	50.8 ± 7.2 (10)	55.4 ± 10.5 (10)	52.4 ± 7.9 (6)
Saline (i.v.)	5 min	8.50 ± 0.19† (6)	43.9 ± 7.8 (5)	47.7 ± 6.2 (6)	34.7 ± 0.7† (4)
Bicuculline	5 min	14.4 ± 0.8†‡ (8)	48.1 ± 8.1 (10)	46.8 ± 5.0* (10)	50.0 ± 14.7 (10)
Diazepam (i.v.) + saline (i.v.)	5 min	9.31 ± 0.89 (6)	40.5 ± 2.2† (5)	40.4 ± 0.5† (5)	37.9 ± 4.3† (5)
Diazepam (i.v.) + bicuculline (i.v.)	5 min	8.46 ± 1.17 (5)	42.2 ± 2.3* (5)	44.1 ± 4.9* (5)	42.5 ± 6.0* (6)

Values are the mean ± s.d. The number of samples are shown in parentheses. Bicuculline dose 0.375 mg/kg, diazepam dose 10 mg/kg, diazepam being given immediately before bicuculline. Results shown different from appropriate untreated rat values: * $P < 0.05$; † $P < 0.01$; or from saline values ‡ $P < 0.01$.

pam (10 mg/kg intravenously) immediately before the bicuculline or saline injection. Diazepam injection alone did not alter either brain or serum calcium concentrations compared to saline-treated rats, with the exception of the hypothalamus (Table 2). The diazepam pretreatment effectively blocked both the convulsant action of the bicuculline and the increase in serum calcium. There were also no changes in regional brain calcium concentration when compared with either saline- or diazepam-injected rats.

Discussion

Reports on the effects of electroconvulsive shock on serum calcium are conflicting. Faragalla & Flach (1970) found that ECT administration produced a prolonged decrease in serum calcium in 3 out of 4 patients and Carman *et al.* (1977) also found decreased serum calcium in 7 patients given ECT, although values were returning towards pretreatment values one week after the last treatment. In contrast, Gour & Chaudhry (1957) noted an immediate and transient hypercalcaemia following ECT. However, no lasting change was observed even after repeated treatment.

In our study, single or repeated ECS produced no marked changes in serum calcium although a small decrease was seen 5 min after a 3 s ECS had been given. In contrast a sustained convulsion produced by bicuculline resulted in a marked hypercalcaemia 5 min after injection. This rise appears to be associated with the muscular changes produced by the central seizure activity, since abolition of the bicuculline-induced convulsion by diazepam pretreatment also abolished the serum calcium changes. The hypercalcaemia seen by Gour & Chaudhry (1957) may also have been produced by muscular activity since it seems unlikely that a muscle relaxant was used in these patients. In addition these authors did not find any change in serum calcium after a course of ECT and our experiments also failed to show any alteration in rat serum calcium concentrations 24 h after repeated ECS. Our data have shown that anaesthesia can produce a significant increase and intravenous injection a decrease in serum calcium concentrations. It is therefore possible that the changes observed by Faragalla & Flach (1970) and Carman *et al.* (1977) may have been produced by the premedication accompanying ECT.

Cardenas & Ross (1975) and Ross (1976) observed large changes in brain calcium (approx. 50%) following administration of various centrally acting drugs. These changes occurred equally in several brain regions including the striatum, hypothalamus and pons/medulla. In contrast, we found that a single ECS or bicuculline-induced seizure produced few significant changes in cerebral calcium concentrations. Nor was any marked change seen in brain calcium when repeated ECS was given at a time when enhanced monoamine-mediated behavioural responses can be demonstrated (Grahame-Smith *et al*, 1978). It has been suggested that the enhanced monoamine-mediated responses might be associated with the antidepressant action of ECT. However, the current data indicate that these enhanced responses do not result from any generalised change in brain calcium concentrations.

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