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The effects of single and repeated electroconvulsive shock administration on the release of 5-hydroxytryptamine and noradrenaline from cortical slices of rat brain

[1]A. Richard Green, [2]David J. Heal & Nigel D. Vincent

MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford OX2 6HE

1 A method is described of measuring the K^+-evoked release of endogenous 5-hydroxytryptamine (5-HT) and noradrenaline (NA) from slices prepared from rat cortex.

2 There was no difference in either the spontaneous (basal) or K^+-evoked release of 5-HT or NA from cortical slices prepared from handled animals and those given a single electroconvulsive shock (ECS) either 30 min or 24 h earlier.

3 In chronic studies, rats were either handled or given an ECS 5 times over 10 days and cortical slices prepared. There was no difference in 5-HT or NA release between the groups 30 min after the last treatment other than a modest attentuation of spontaneous NA release following ECS treatment. However 24 h after the last treatment K^+-evoked release (above basal release) of 5-HT and NA was inhibited by 84% and 48%, respectively.

4 These data demonstrate that following a single ECS, normal 5-HT and NA release is seen at a time when GABA release is markedly inhibited. After repeated ECS the release of both monoamines was markedly inhibited. These 5-HT changes may be involved in the enhanced 5-HT-receptor function seen after repeated ECS.

Introduction

In the study by Nutt *et al.* (1981) on the rise in seizure threshold following a convulsion, it was proposed that there was a neurotransmitter selective change in γ-aminobutyric acid (GABA) function acid following a convulsion. Subsequent studies (Bowdler & Green, 1982; Green *et al.*, 1987a,b) have demonstrated marked changes in GABA synthesis and release following a convulsion. What has not been demonstrated, however, is the specificity of this change and whether the inhibition of release is particular to GABA or merely the reflection of a general change in brain biochemistry; for example a perturbation of membrane function.

The current study has, therefore, examined the effect of a seizure induced by electroconvulsive shock (ECS) on the release of endogenous 5-hydroxytryptamine (5-HT) and noradrenaline (NA) from slices prepared from rat frontal cortex.

[1] Author for correspondence; present address: Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ.

[2]Present address: The Boots Co: plc, Research Department, Nottingham NG2 3AA.

Repeated (ECS) has also been shown to alter GABA synthesis and release (Green *et al.*, 1978; Green & Vincent, 1987). A study has, therefore, also been made of 5-HT and NA release from cortical slices both 30 min and 24 h after the last of a series of 5 ECS, given spread out over 10 days, in a manner analagous to the clinical administration of electroconvulsive therapy (ECT).

Methods

Animals and ECS administration

Male Sprague-Dawley derived rats (Charles River, Kent) weighing 120–150 g were used. They were housed in groups in conditions of controlled temperature (21 ± 1°C) and lighting (light period 07 h 00 min–19 h 00 min) and food (modified 41B pellets) and tap water were available *ad libitum*.

Electroconvulsive shocks were administered

through ear-clip electrodes using a Theratronics small animal electroplexy unit (120 V, 1s, 50 Hz sinusoidal). Rats were either given a single ECS or 5 ECS spread out over 10 days (Monday, Wednesday, Friday, Monday, Wednesday). Control animals were handled, the ear-clips placed but no current passed.

Measurement of endogenous 5-HT and NA from slices of rat frontal cortex

Rats were killed by pneumothoracic stun and decapitation, and the frontal cortex dissected out on an iced surface. Tissue slices were prepared by chopping on a McIlwain tissue chopper in two directions at 45° at 250 μm intervals, and were immediately suspended in 0.5 ml of a freshly gassed (95% O_2, 5% CO_2) calcium-poor Krebs bicarbonate buffer (composition: NaCl 126 mM, KCl 1.8 mM, KH_2PO_4 1.24 mM, $MgSO_4$ 1.3 mM and pargyline, 50 μM according to the modified method of Bennett *et al.* (1982). The initial washing procedure in a Ca^{2+}-poor medium prevents considerable loss of the analyzed neurotransmitters from the tissue slice preparation (Bennett, personal communication). Four tubes containing approximately 15–20 mg of frontal cortical tissue each (being tissue derived from two control and two ECS-treated animals) were incubated for 10 min at 37°C before centrifugation at 1000 *g* for 30 s.

The tissue slices were then resuspended in 0.5 ml of freshly gassed (95% O_2, 5% CO_2) normal Krebs bicarbonate buffer, including calcium chloride (2.4 mM), glucose (10 mM) fluoxetine (50 μM) and pargyline (50 μM), for 25 min at 37°C. Five minutes into this incubation period, 10 μl of a high K^+ solution (final concentration 35 mM) was added to one tube from each of the two groups. The remaining tube from each group received 10 μl of normal buffer, and incubation was continued for a further 20 min before termination by centrifugation for 30 s at 1000 *g*.

Two samples of 200 μl of supernatant were removed from each sample and each added to 20 μl of a 1.0 M perchloric acid containing 4.0 mM sodium metabisulphate (antioxidant) solution in Eppendorf tubes. One aliquot for assay of 5-HT content was stored on ice, and one for NA assay was stored frozen at −70°C from each supernatant sample. Analysis of 5-HT and NA content was performed separately by h.p.l.c. with electrochemical detection as described below. For all experiments, paired values were thus obtained for the spontaneous efflux and K^+-evoked release of endogenous neurotransmitter from brain slices prepared from two control and two ECS-treated animals. The method thus allowed for the measurement of the evoked release of both endogenous 5-HT and NA from the same tissue slice preparation.

Measurement of 5-HT and NA by high performance liquid chromatography

Supernatants obtained from tissue slice experiments were taken off ice, or thawed (in the case of those samples for NA determination), centrifuged at 1000 *g* for 2 min before analysis to remove the presence of any protein in the sample and analysis of 5-HT and NA carried out on two separate h.p.l.c. systems.

The mobile phase employed for 5-HT determination was 0.1 M sodium acetate-citric acid, pH 4.0, containing 19% (v/v) methanol pumped at a rate of 1.2 ml min^{-1}. Separation was obtained using a Spherisorb ODS 5 analytical column, 25 cm × 4.6 mm (i.d.). Detection was by means of an BAS LC-4 amperometric detector containing a TL-5 flow cell with a glassy carbon electrode maintained at a potential 0.7 V vs. a Ag/AgCl, reference electrode (Bioanalytical Systems Inc., West Lafayette, U.S.A.).

The mobile phase for NA was a 0.1 M sodium acetate-citric acid buffer, pH 4.0, containing 22% methanol (v/v), with 5 mM octane sulphonic acid (sodium salt), pumped at a rate of 0.8 ml min^{-1}. The analytical column for NA was an Altex Utrasphere ODS 5 25 cm × 4.6 mm (i.d.). Detection of NA was achieved with a BAS LC-4B Amperometric detector fitted with a TL-5 flow cell with a glassy-carbon electrode held at a potential 0.6 V vs. a Ag/AgCl reference electrode.

Samples (100 μl) for the estimation of 5-HT and NA were run on these systems in duplicate following the standardization of the systems with known concentrations of 5-HT and NA, made fresh from stock solutions before use. Verification of those peaks obtained for endogenous 5-HT and NA from experimental samples was performed by injection of 50 μl samples with 50 μl of a known concentration of 5-HT and NA and observing the coincidence and height of peaks upon recording. The presence of fluoxetine in the incubation medium increased the concentration of 5-HT for both the spontaneous efflux and K^+-evoked release of endogenous 5-HT by 5–6 fold, without affecting the measured levels of endogenous NA (unpublished observations).

Results

The effect of a single ECS on the release of endogenous 5-HT and NA

Rats were either handled or given a single ECS, and killed either 30 min or 24 h later. Brain slices were prepared and the release of endogenous 5-HT and NA measured as described in the Methods. Thirty min after the seizure no difference in either the spontaneous or K^+-evoked release of 5-HT and NA in the

Table 1 Potassium evoked release of endogenous noradrenaline and 5-hydroxytryptamine in cortical slices 30 min after a single electroconvulsive shock (ECS)

	Endogenous transmitter released (pmol mg^{-1} tissue per incubation)	
Treatment	*Handled*	*ECS*
5-Hydroxytryptamine		
Spontaneous	6.14 ± 0.63 (5)	6.40 ± 0.64 (5)
K^+ (35 mM)	21.18 ± 1.84 (5)	23.77 ± 1.20 (5)
Noradrenaline		
Spontaneous	0.54 ± 0.19 (5)	0.71 ± 0.37 (6)
K^+ (35 mM)	3.71 ± 1.65 (6)	3.66 ± 1.50 (6)

Results expressed as mean ± s.d. with number of observations in parentheses.

ECS-treated group was observed compared to handled controls (Table 1). Similarly no change was observed in release perameters 24 h after the ECS (data not shown).

The effect of repeated ECS administration on the release of endogenous 5-HT and NA, 30 min after the final seizure

Rats were either handled or given ECS 5 times over 10 days, and killed 30 min after the final treatment. There was no difference in the spontaneous or K^+-evoked release of endogenous 5-HT between ECS-treated and handled controls (Table 2).

Repeated handling significantly ($P < 0.02$) elevated the spontaneous efflux of NA from frontal cortical slices compared to those animals that had received a single handling treatment (compare Table 2 with Table 1) an effect which was apparently decreased by ECS administration (Table 2).

However, there was no significant difference in the K^+-evoked release of NA between ECS-treated animals and the control group although the mean value was lower in the ECS-treated group (Table 2). The percentage increase in the release of NA with K^+ above basal efflux for both control and ECS-treated animals was similar.

The effect of repeated ECS administration on the release of endogenous 5-HT and NA, 24 h after the final seizure

Rats were either handled or given ECS 5 times over 10 days, and killed 24 h later. There was no difference in the spontaneous efflux of endogenous 5-HT between the control and ECS-treated groups 24 h after repeated treatment (Table 3). However, a significant reduction in the K^+-evoked release of 5-HT was observed in the ECS-treated group at this time (Table 3).

Table 2 The effect of repeated electroconvulsive shocks (ECS) on potassium-evoked release of endogenous noradrenaline and 5-hydroxytryptamine studied 30 min after the final shock

	Endogenous transmitter released (pmol mg^{-1} tissue per incubation)	
Treatment	*Handled*	*ECS*
5-Hydroxytryptamine		
Spontaneous	6.80 ± 1.60 (8)	7.24 ± 0.48 (9)
K^+ (35 mM)	21.05 ± 1.20 (8)	24.00 ± 2.8 (8)
Noradrenaline		
Spontaneous	1.20 ± 0.38 (7)	0.84 ± 0.21 (9)*
K^+ (35 mM)	4.46 ± 1.69 (7)	3.71 ± 1.28 (9)

Results expressed as mean ± s.d. with number of observations in parentheses.
* Different from spontaneous release in handled rats, $P < 0.05$.

Table 3 The effect of repeated electroconvulsive shocks (ECS) on potassium-evoked release of endogenous noradrenaline and 5-hydroxytryptamine studied 24 h after the final shock

	Endogenous transmitter released (pmol mg^{-1} tissue per incubation)	
Treatment	*Handled*	*ECS*
5-Hydroxytryptamine		
Spontaneous	6.28 ± 2.43 (10)	8.14 ± 3.52 (10)
K^+ (35 mM)	19.91 ± 4.08 (8)	10.34 ± 2.79 (9)**
Noradrenaline		
Spontaneous	0.96 ± 0.33 (10)	0.70 ± 0.28 (7)
K^+ (35 mM)	4.20 ± 1.60 (9)	2.41 ± 0.48 (6)*

Results expressed as mean ± s.d. with number of observations in parentheses. * Different from K^+-evoked release in handled rats, $P < 0.01$.

Repeated handling or ECS also had no effect on the spontaneous efflux of endogenous NA from slices of frontal cortex 24 h after treatment, but there was a significant inhibition in the K^+-evoked release of endogenous NA in ECS-treated animals (Table 3).

Discussion

The method presented in this paper allows for the determination of both endogenous 5-HT and NA release from the same tissue preparation. This method also possesses considerable advantages over those previously employed by virtue of its comparative simplicity (for example, with respect to a superfusion system of analysis), and its ability to measure the release of endogenous, as opposed to preloaded, radiolabelled transmitters. The spontaneous efflux of these transmitters, which is as low as 0.5–1.20 pmol mg^{-1} tissue $20\ min^{-1}$ for NA, and between approximately 6–8 pmol mg^{-1} tissue $20\ min^{-1}$ for 5-HT, are detectable with h.p.l.c. with electrochemical detection.

Whilst there are methodological advantages to using fixed volume release studies both in terms of simplicity and the ability to examine release of endogenous transmitter, there are nevertheless also distinct drawbacks in the method. The major problem is that amine released into the medium accumulates and can thus be subject to re-uptake. More seriously, the concentration in the medium may influence feedback mechanisms by acting at presynaptic autoreceptors or heteroceptors. A change in the function of these receptors may therefore influence the result obtained.

The addition of a high K^+ medium (35 mM) to the incubate resulted in a large and significant increase in the release of both transmitters, of the order of 350% above baseline efflux for 5-HT and approximately 650% for NA. The ratio of the K^+-evoked to spontaneous release of transmitter is similar to that previously observed using superfusion of cortical slices preloaded with either [^{3}H]-5-HT or [^{3}H]-NA (Minchin *et al.*, 1983) while the percentage stimulation of release with K^+ was lower in the earlier work. However, exposure to the K^+ depolarization stimulus was for a 3 min period in the superfusion study as opposed to a 20 min incubation period in this study.

There was no statistical difference in the spontaneous efflux of 5-HT and NA from animals which had received a single ECS and were killed 30 min or 24 h later, compared with handled controls. The K^+-evoked release of endogenous 5-HT and NA from slices of frontal cortex was also not statistically different for either group. These data agree with the previous work of Minchin *et al.* (1983) employing a superfusion system containing slices of cerebral cortex preloaded with [^{3}H]-5-HT or [^{3}H]-NA from animals which received either a single ECS with halothane, or halothane alone.

The principal significance of the data obtained after a single ECS in this study, is that a normal K^+-evoked release of endogenous NA and 5-HT was demonstrated at a time after a single ECS when the release of endogenous GABA was significantly inhibited (Green *et al.*, 1987b). These results would tend to indicate the selectivity of the changes in GABAergic biochemistry following ECS, since the inhibition of endogenous GABA release does not appear to be merely a reflection of a more widespread inhibition of transmitter release in the rat brain.

The investigation of the actions of repeated ECS administration show that spontaneous and K^+-evoked release of endogenous 5-HT from frontal cortical slices was not different between handled and ECS-treated animals 30 min after the last seizure compared with data obtained after a single ECS. Repeated handling did, however, significantly

increase the spontaneous release of endogenous NA. This may be a stress-related phenomenon because repeated anaesthetic or saline injection has previously been observed to alter NA turnover when compared with a single treatment (Nimgaonkar *et al.*, 1986). Nevertheless, this change was not observed 24 h later suggesting that it may be a short-lived phenomenon and not a longer adaptation in NA release in response to chronic handling. No significant difference in the K^+-evoked release of NA was found between control and ECS-treated animals 30 min after the last ECS, whether expressed as the percentage increase in release above basal efflux for the two groups (data not shown) or as the mean values for K^+-evoked release (see Table 2).

An inhibition of the K^+-evoked release of both NA and 5-HT was seen 24 h after repeated ECS treatment. In percentage terms, the inhibition of the K^+-evoked release above spontaneous efflux compared with handling controls was larger for 5-HT (84%) than for NA (47%), while basal efflux was unaltered by ECS.

These findings are in contrast to those of Minchin *et al.* (1983) who failed to observe any alteration in the evoked release of the radiolabelled transmitters from preloaded cortical slices. This discrepancy between the two methods of analysing transmitter release is clearly similar to that demonstrated for endogenous and [^{3}H]-GABA, following a single ECS (Green *et al.*, 1987b) and strengthens the view that the preloaded radiolabel may fail to exchange evenly with endogenous pools for the transmitter and thus become associated with compartments from which release in the presence of a physiological stimulus is not typical (see Herdon *et al.*, 1985; Green *et al.*, 1987b).

The current data suggest that following repeated ECS there is an inhibition of 5-HT release from terminals in the cortex. Repeated ECS results in an increase in the number of post-synaptic 5-HT_2 receptors (Kellar *et al.*, 1981; Green *et al.*, 1983; Goodwin *et al.*, 1984) and drugs shown to inhibit 5-HT release do increase cortical 5-HT_2 receptor density in the cortex (Green *et al.*, 1985; Gray *et al.*, 1986). It is therefore possible that the changes seen in the current study and the 5-HT_2 receptor change seen after repeated ECS are related.

The current data also demonstrate that sustained changes in NA release occur after repeated ECS and these results are more difficult to equate with observed changes in NA biochemistry and function. Repeated ECS increases both K_m and V_{max} for [^{3}H]-NA uptake into cortical slices (Hendley, 1976; Minchin *et al.*, 1983). Both β-adrenoceptor (Bergstrom & Kellar, 1979; Nimgaonkar *et al.*, 1986) and α_2-adrenoceptor number (Stanford & Nutt, 1982) and function (Heal *et al.*, 1981) have been shown to decrease after repeated ECS. Whether these several changes are in any way related must await further study.

N.D.V. held a M.R.C. studentship during this study.

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