Studies on $[3^\mathrm{H}]$Diazepam and $[3^\mathrm{H}]$Ethyl-β-Carboline Carboxylate Binding to Rat Brain In Vivo. II. Effects of Electroconvulsive Shock

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Abstract: In vivo specific binding of $[3^\mathrm{H}]$diazepam was not altered by a single electroconvulsive shock given 5, 30, or 60 min, or 24 h previously, nor 24 h after the last of 10 daily shocks. Similarly, in vivo $[3^\mathrm{H}]$ethyl-β-carboline carboxylate binding was not changed in the brains of animals that had been given a single electroconvulsive shock 30 min previously or a series of 10 daily shocks. Brain areas examined included cerebral cortex, hippocampus, cerebellum, and striatum. However, cortical binding of $[3^\mathrm{H}]$diazepam was increased by 32% in animals which were present in the same room while another was being injected and killed. This may represent a response to stress and/or anxiety. Key Words: Benzodiazepines—β-Carbolines—In vivo binding—Electroconvulsive shock. Nutt D. J. and Minchin M. C. W. Studies on $[3^\mathrm{H}]$diazepam and $[3^\mathrm{H}]$ethyl-β-carboline carboxylate binding to rat brain in vivo. II. Effects of electroconvulsive shock. J. Neurochem. 41, 1513–1517 (1983).

In a companion paper (Minchin and Nutt, 1983) we described some characteristics of the binding of both diazepam and ethyl-β-carboline carboxylate (β-CCE) to rat brain in vivo. We have now examined whether single or repeated electroconvulsive shock (ECS) alters the in vivo binding of either of these ligands. There have been a number of studies of the effects of ECS on in vitro benzodiazepine binding. Following a single ECS, Paul and Skolnick (1978) reported increased $[3^\mathrm{H}]$diazepam binding to rat cortical membranes. This increase was not seen until 15 min after the seizure and had disappeared by 60 min. However, a recent study has not confirmed these findings (Bowdler and Green, 1982). Repeated seizures have also been reported to change benzodiazepine binding in vitro. McNamara et al. (1980) showed that $[3^\mathrm{H}]$diazepam binding to hippocampal membranes was increased after 17 daily ECSs. Subsequently this group demonstrated in slice preparations that the increase in hippocampal benzodiazepine binding was confined to the dentate granule cells (Valdes et al., 1982). In contrast, Bowdler et al. (1983) reported no change in either cortical or hippocampal $[3^\mathrm{H}]$diazepam binding following a course of 10 daily ECSs.

These apparent discrepancies may relate to differences in the levels of endogenous substances that affect benzodiazepine receptor binding in the various in vitro preparations. One such substance, γ-aminobutyric acid (GABA), has been shown to increase in concentration following both a single and repeated ECS (Green et al., 1978; Bowdler and Green, 1982; Bowdler et al., 1983). Repeated experiments have shown that GABA increases benzodiazepine binding in vitro (Olsen, 1981), and other endogenous substances may also modify benzodiazepine binding (Guidotti et al., 1978; Marangos et al., 1978; Asano and Spector, 1979; Rommel-spacher, 1981). Since these substances may be removed to varying degrees in in vitro preparations, such binding experiments may not reflect alterations in the state of the benzodiazepine receptor in vivo following ECS. We have accordingly addressed this question by the use of in vivo benzodiazepine receptor ligand binding techniques.

METHODS

ECS was administered to unanaesthetised rats by means of carclip electrodes. A constant-voltage device delivered
125 V with a 50-Hz sinusoidal waveform for 1 s. Handled controls had the earclips applied, but no current was passed.

In vivo binding was measured exactly as described in the accompanying paper (Minchin and Nutt, 1983). Initially, ECS-treated and control animals were treated in pairs; each animal was placed in a restraining tube, injected i.v. with "H ligand, and killed in the same room. However, it became clear that in these circumstances the second animal in each pair showed markedly increased 

[3H]diazepam binding in the cerebral cortex (see Results), regardless of prior treatment. This effect could be abolished if each rat in a pair was injected and killed in a separate room, to which it had been acclimatised. Accordingly, all subsequent experiments were performed in this way, including all the beta-CCE binding estimations.

In experiments designed to measure protein entrapment on the filters, homogenates from ECS-treated and control rats were prepared in the usual way. Portions of 200 µl were filtered and the filters were rinsed twice with 5 ml ice-cold Tris-HCl buffer (50 mM, pH 7.4). The filters were extracted by heating them with 1.5 ml 1 M NaOH at 50°C for 2 h. Portions of the extract and portions of the whole homogenate were assayed for protein content by the method of Lowry et al. (1951).

RESULTS

Animals which had been waiting in the same room while the other member of the pair was being injected and killed showed a 32% increase in in vivo 

[3H]diazepam binding in the cerebral cortex (Table 1). Binding in the hippocampus and cerebellum was not altered, and the effect disappeared when the animals were acclimatised, injected, and killed in separate rooms. Subsequent experiments were therefore performed on separated pairs of rats.

ECS given 5 min before injection of 

[3H]diazepam significantly increased the total amount of label entering the cortex and hippocampus (Fig. 1); this effect was not evident 30 min after a single ECS, 24 h after a single shock, or 24 h after 10 daily shocks. At 1 h after ECS there was a slight increase in the amount of 

[3H]diazepam in the cortex, but not the hippocampus. ECS did not change the entry of 

[3H]beta-CCE in either cortex or hippocampus when given once 30 min before injection or when given daily for 10 days 24 h before injection (Fig. 2). As stated in the companion paper (Minchin and Nutt, 1983), the amount of specific 

[3H]diazepam or 

[3H]beta-CCE bound in vivo increased linearly with the free concentration of the ligands. ECS did not alter this relationship; therefore, all the data could be normalised with respect to the amount of radioactivity present in the homogenate. When this was done, it became clear that none of the ECS schedules changed specific 

[3H]diazepam binding in any of the regions examined (Table 2). Similarly, specific in vivo 

[3H]beta-CCE binding was unaltered after a single shock or 24 h after the last of 10 daily shocks (Table 3). However, the hippocampal control values 30 min after a sham shock were significantly lower than those of unhandled controls (14.4 ± 0.06, n = 4, p < 0.05, two-tailed t-test). Control hippocampal binding 24 h after 10 daily sham ECS treatments was higher than unhandled control values, but the difference did not reach significance.

The possibility was considered that ECS may alter the physical characteristics of the membranes, but if this occurred, it was not reflected in a change in the ability of GF/B filters to entrap them (Table 4).

DISCUSSION

The increase in cortical 

[3H]diazepam binding seen in animals which are witness to the experimental handling of another animal is the first in vivo evidence, albeit serendipitous, that the benzodiazepine system in the CNS is capable of alteration in response to physiological stimuli. The nature of the stimulation in the present experiments is unclear, but is possibly related to stress or, perhaps more likely, anxiety. The change underlying the increase in cortical binding may represent a rapid (within 10 min) increase in the number of binding sites possibly involving the unmasking of hitherto unavailable sites, since de novo synthesis seems unlikely within such a short time span. This suggestion gains some support from the experiments of Soubrie et al. (1980), in which a cold water swim (6°C) increased the number of cerebral (but not cerebellar) 

[3H]flunitrazepam binding sites in vitro. However,

| TABLE 1. Effect of the order of injection on in vivo 

[3H]diazepam binding |
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<tr>
<td>Specific [3H]diazepam binding</td>
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<tr>
<td>Injected 1st</td>
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<td>Injected 2nd</td>
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Animals were treated in pairs, being injected with [3H]diazepam and killed one after the other in the same room. The time interval between injection of the first and second rat was approximately 10 min. Specific [3H]diazepam binding was measured as described in Methods. Each value is the mean ± SEM, with the number of animals in parentheses, binding in each animal being determined in duplicate.

* p < 0.01, 2-tailed t-test.
BENZODIAZEPINE BINDING AND ELECTROCONVULSIVE SHOCK

FIG. 1. [3H]Diazepam levels in brain homogenates following i.v. injection of [3H]diazepam and the effects of ECS. Animals that had been either sham-shocked or given a single ECS were injected at various times afterwards with 40 μCi [3H]diazepam and killed 2 min later. Rats given 10 once-daily shocks were injected 24 h after the last shock. The brains were dissected, frozen, and subsequently homogenised as described in Methods. Portions of the homogenate were counted, values for each animal being represented by a dot. Horizontal lines are the means, and p values (2-tailed t-test) are given below each ECS-treated group. N.S. = Not significant.

Le Fur et al. (1979) found no change in either in vitro specific [3H]diazepam binding or in vivo total [3H]flunitrazepam binding in the brains of rats subjected to a 5-min swim at 20°C, despite the elevated plasma corticosteroid levels which this treatment evoked. This suggests that stress alone was not responsible for the change in benzodiazepine binding observed in the present study. It is equally possible that the stimulus in the present experiments caused a reduction in the levels of an endogenous cortical ligand, thus permitting increased binding of the exogenous [3H]diazepam. Further experiments are necessary to clarify this point.

The increase in the amount of [3H]diazepam entering the brain 5 min after an ECS is probably the result of the increased cerebral blood flow that is associated with seizures (Horton et al., 1980; Ingvar et al., 1981). By 30 min, however, this effect was no longer apparent.

[3H]Diazepam binding in vivo was unaltered at several time points after a single ECS in all three brain regions examined. This supports the in vitro
findings of Bowdler and Green (1982), who did, however, describe an increase in the GABA content of several brain regions following a single shock, with the rise in hippocampal GABA having a similar time course to the increase in seizure threshold which occurs postictally. The present results suggest that this phenomenon is not linked to an alteration in benzodiazepine receptor sensitivity, although GABA is known to reduce the $K_d$ of benzodiazepine binding in vitro (Tallman et al., 1978). It is possible that the elevation of GABA levels occurs in a compartment that does not have access to benzodiazepine receptors. Furthermore, if a seizure changes the level of any other endogenous effector substance, it apparently does so in a way that leaves the benzodiazepine receptor unaffected. We can also conclude that there is no detectable change in the benzodiazepine receptor itself, that is, in its $K_d$ or $B_{\text{max}}$.

Similar considerations apply in the analysis of the multiple ECS experiments. Here striatal GABA levels have been shown to be elevated 24 h after the last of 10 once-daily shocks (Bowdler et al., 1982), a protocol known to result in pronounced behavioural effects (Green, 1980); again this apparently had no effect upon diazepam binding. Neither could we find any change in binding in the hippocampus, which implies either that our ECS protocol was sufficiently different from that of McNamara et al. (1980) for the changes they observed in vitro not to occur or that the results of multiple ECS only become apparent during extraction and preparation of brain membranes.

$[^3\text{H}]\beta$-CCE has some selectivity for the $BZ_1$ subtype of benzodiazepine receptor (see Minchin and Nutt, 1983), yet in vivo binding of this ligand was not affected by single or multiple ECS in any of the brain regions examined. Functional changes in endogenous ligands or the receptors themselves as a result of ECS seem, therefore, to be ruled out for this system also. However, there was some indication that hippocampal $[^3\text{H}]\beta$-CCE binding was sensitive to the handling procedures used during sham ECS. There was also a slight variation in the various handled controls for $[^3\text{H}]$diazepam binding in the cortex and hippocampus (see Table 2). This, combined with the injection order effect on cortical $[^3\text{H}]$diazepam binding, suggests that although ECS does not appear to alter the benzodiazepine binding system in vivo, stressful and/or anxiogenic stimuli of a different nature may indeed have such an effect.

### Table 2. Lack of effect of ECS on in vivo $[^3\text{H}]$diazepam binding

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Cerebellum</th>
<th>Striatum</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.4 ± 1.1 (12)</td>
<td>26.1 ± 1.4 (4)</td>
<td>25.1 ± 1.0 (11)</td>
<td>—</td>
</tr>
<tr>
<td>5 min after ECS × 1</td>
<td>26.2 ± 1.2 (4)</td>
<td>26.1 ± 1.4 (4)</td>
<td>25.9 ± 1.1 (4)</td>
<td>—</td>
</tr>
<tr>
<td>30 min after ECS × 1</td>
<td>27.8 ± 1.1 (9)</td>
<td>27.0 ± 0.7 (9)</td>
<td>23.6 ± 0.9 (9)</td>
<td>—</td>
</tr>
<tr>
<td>60 min after ECS × 1</td>
<td>24.2 ± 1.9 (4)</td>
<td>24.7 ± 0.4 (4)</td>
<td>26.3 ± 1.5 (4)</td>
<td>—</td>
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<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Cerebellum</th>
<th>Striatum</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.3 ± 1.0 (3)</td>
<td>22.1 ± 0.9 (3)</td>
<td>24.8 ± 2.8 (3)</td>
<td>—</td>
</tr>
<tr>
<td>24 h after ECS × 1</td>
<td>24.3 ± 3.5 (3)</td>
<td>23.7 ± 2.4 (3)</td>
<td>21.2 ± 1.0 (3)</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>28.7 ± 1.6 (8)</td>
<td>28.5 ± 1.3 (8)</td>
<td>25.5 ± 1.4 (8)</td>
<td>24.4 ± 1.1 (4)</td>
</tr>
<tr>
<td>24 h after ECS × 10</td>
<td>31.5 ± 1.1 (7)</td>
<td>27.9 ± 2.0 (8)</td>
<td>27.6 ± 0.8 (7)</td>
<td>24.6 ± 1.5 (4)</td>
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</table>

Controls for the acute experiments were not different from one another, nor from naive controls, and were therefore pooled. Each animal was either handled or given ECS and injected with $[^3\text{H}]$diazepam at various times afterwards. The values are the mean ± SEM of the number of experiments in parentheses, each experiment being determined in duplicate.

### Table 3. Lack of effect of ECS on in vivo $[^3\text{H}]\beta$-CCE binding

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Cerebellum</th>
<th>Striatum</th>
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<tbody>
<tr>
<td>Control</td>
<td>20.5 ± 1.4 (6)</td>
<td>10.8 ± 1.0 (6)</td>
<td>17.3 ± 2.0 (4)</td>
<td>10.3 ± 0.8 (5)</td>
</tr>
<tr>
<td>30 min after ECS × 1</td>
<td>22.1 ± 2.0 (5)</td>
<td>13.1 ± 0.8 (5)</td>
<td>15.0 ± 3.4 (5)</td>
<td>8.4 ± 0.7 (5)</td>
</tr>
<tr>
<td>Control</td>
<td>22.8 ± 2.6 (5)</td>
<td>19.2 ± 2.9 (5)</td>
<td>20.5 ± 1.4 (5)</td>
<td>9.3 ± 2.6 (4)</td>
</tr>
<tr>
<td>24 h after ECS × 10</td>
<td>26.1 ± 1.7 (6)</td>
<td>17.8 ± 2.5 (4)</td>
<td>20.3 ± 2.9 (3)</td>
<td>12.4 ± 2.5 (4)</td>
</tr>
</tbody>
</table>

Animals were handled or given ECS and injected with $[^3\text{H}]\beta$-CCE either 30 min after 1 shock or 24 h after 10 once-daily shocks. The values are means ± SEM, with the number of animals in parentheses. The binding in each animal was determined in duplicate.
TABLE 4. Protein entrapment on filters

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
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<th>Cerebellum</th>
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<tbody>
<tr>
<td>Homogenate protein on filter</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control 30 min after ECS</td>
<td>74.5 ± 5.7 (4)</td>
<td>72.0 ± 6.9 (4)</td>
<td>65.4 ± 5.2 (4)</td>
</tr>
<tr>
<td>ECS × 1</td>
<td>69.8 ± 4.2 (4)</td>
<td>65.6 ± 4.4 (4)</td>
<td>69.0 ± 6.8 (3)</td>
</tr>
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* Rats were either handled or given a single ECS and killed 30 min later. Homogenates were prepared in the same way as for benzodiazepine binding experiments and portions filtered and rinsed. The protein on the filters and in the homogenates was estimated, and the former expressed as a percentage of the latter. Each value is the mean ± SEM of the number of experiments in parentheses.

REFERENCES


