CHANGES IN SENSITIVITY OF MICE TO ANTICONVULSANT DRUGS FOLLOWING BILATERAL OLFACTORY BULB ABLATIONS

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Accepted September 29, 1976

Abstract—Changes in sensitivity to anticonvulsant drugs were investigated after bilateral olfactory bulb ablations in mice. The sensitivity to benzodiazepines and acetazolamide increased, whereas that to phenylacetylurea and dipropylacetic acid decreased, and sensitivity to phenobarbital, diphenylhydantoin and trimethadion was not significantly changed after olfactory bulb ablations. Increase in sensitivity to benzodiazepines was the most significant in both electroshock and pentetrazol convulsions. It was suggested that altered activities and denervation supersensitivity in the limbic system, hypothalamus and midbrain might account for these changes in sensitivity to anticonvulsant drugs after olfactory bulb ablations.

In a previous study (1), it was found that susceptibility of mice to convulsion induced by either electroshock or CNS stimulants was greatly changed after bilateral ablations of the olfactory bulbs, with concomitant appearance of hyperactivity and hyperreactivity.

The present investigation was therefore an attempt to determine if any changes in anticonvulsant effects of various antiepileptic drugs occurred in mice following bilateral ablations of the olfactory bulbs.

MATERIALS AND METHODS

Animals: Male CF1 mice weighing 20–28 g, supplied from the Kyushu University Institute of Experimental Animals, were used. Bilateral ablation of the olfactory bulbs was performed by suctioning, as described in the previous study (1). After termination of the experiments, the whole brain was removed, frozen-tissue preparations were made, and the extent of olfactory bulb lesions was verified histologically. Mice in which more than 1/3 of the olfactory bulb on either side remained intact or in which there were any injuries in the frontal cortex, were discarded. The mice with bilateral ablation of the olfactory bulb (O.B. mice) were used for experiments from 10 days after the surgery.

As a control group, intact mice were used, instead of sham-operated ones, because no difference was found in susceptibility to convulsion between intact and sham-operated mice (1).

Measurement of anticonvulsant effects: The protective effects on both maximal electroshock (MES) and pentetrazol convulsions were examined. In some experiments, minimum electroshock threshold (MET) was also measured.

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To induce MES convulsion, sinusoidal A.C. current of 60 Hz and 50 mA was applied to the head of mice through corneal electrodes (1), using the Woodbury and Davenport apparatus (2) with some modification. Prior to the drug tests, animals were subjected twice to the experience of MES convulsion, as many mice died during their first such experience. As an index of the anticonvulsant effect of MES convulsion, a loss of the tonic extensor phase (TE) was used. In MET test, a minimum electric current sufficient to induce minimal full seizure was measured, and a 50% convulsant current was determined.

In the pentetrazol test, 85 mg/kg of pentetrazol was injected s.c. at a peak time of the drug effect, and a suppression of clonic convulsion was used as an index of anticonvulsant effect.

**Statistical analysis:** Eight animals each of both the intact and O.B. mice were used for each dose of the test drugs. All the data were statistically analysed and the ED50 was calculated using Litchfield-Wilcoxon's method.

**Drugs:** Test drugs used in this experiment were chlordiazepoxide (Contol, Takeda Yakko Co.), diazepam (Cercine, Takeda Yakko Co.), nitrazepam (Benzalin, Shionogi Seiyaku Co.), phenobarbital sodium (Fujinaga Seiyaku Co.), diphenylhydantoin sodium (Aleviatin Sodium, Dainippon Seiyaku Co.), trimethadion (Minoaleviatin, Dainippon Seiyaku Co.), acetazolamide (Diamox, Lederle Co.), phenylacetylene (Phenurone, Dainippon Seiyaku Co.), and dipropylacetic acid (Shionogi Seiyaku Co.).

All drugs were dissolved in physiological saline solution and injected s.c. except for nitrazepam and phenylacetylene which were suspended in a 0.5% carboxymethylcellulose (CMC) solution and given orally via stomach tube.

**RESULTS**

**Effects of benzodiazepines**

**Chlordiazepoxide (CDP):** Effects of chlordiazepoxide (CDP) on the MES convulsion were compared between the O.B. and normal mice. MES was applied 120 min after s.c. administration of CDP. The anticonvulsant effect of CDP is shown in Table 1, in terms of the number of deaths after pentetrazol convulsion.

| dose (mg/kg s.c.) | MES convulsion | dose (mg/kg s.c.) | pentetrazol convulsion |
|------------------|----------------|------------------|-----------------------|
|                  | intact         | O.B.             | intacht                | O.B.                  |
| 10               | 1/8            | 2                | 2/8 (0)               |
| 20               | 4/8            | 3                | 3/8 (0)               |
| 50               | 6/8            | 5                | 1/8 (0)               |
| 70               | 2/8            | 7                | 4/8 (0)               |
| 100              | 5/8            | 10               | 6/8 (0)               |
| 120              | 6/8            |                  |                       |

ED50 (mg/kg) (95% confidence limits) 96.8 (79.2-114.4) 27.4 (14.1-37.8) 7.6 (5.6-9.2) 3.6 (2.2-5.1)

(1): the number of deaths after pentetrazol convulsion.
of incidence of protection (TE loss). CDP was administered at doses from 70 to 120 mg/kg in the intact and from 10 to 50 mg/kg in the O.B. mice in order to obtain the ED50 for protection against MES convulsion, because we found that CDP was much more effective in the O.B. mice than in the intact animals. The ED50 of CDP in protecting against MES convulsion in the intact mice was approx. 3.5 times greater than that in the O.B. mice. It was also evident that effects of CDP on behavior such as ataxia and muscular relaxation were more marked in the O.B. mice than in the intact animals.

In the pentetrazol convulsion test, pentetrazol was injected s.c. at a dose of 85 mg/kg 110 min after s.c. administration of CDP. CDP was approx. twice as effective in the O.B. mice as in the intact group (Table 1).

**Diazepam (DZP):** MES convulsion was tested 30 min after s.c. administration of diazepam (DZP), and pentetrazol was injected 20 min after DZP. The results are shown in Table 2 as well as in Figs. 1–2. DZP was much more effective in the O.B. mice than in the intact group both on MES and pentetrazol convulsions. Ataxia and muscular relaxation were also more marked in the O.B. mice.

The effect of DZP on MET was also tested in the O.B. mice and compared with that in intact mice (Table 3). The 50% convulsant electric current (CD50) in MET test was 7.1

**Table 2. Comparison of anticonvulsant effects of diazepam in intact and O.B. mice**

| dose (mg/kg s.c.) | MES convulsion | pentetrazol convulsion |
|-------------------|----------------|------------------------|
|                   | intact | O.B. | dose (mg/kg s.c.) | intact | O.B. |
| 2                 | 2.8    |     | 0.2               | ---    | 2.8 (2) |
| 5                 | 4.8    |     | 0.3               | ---    | 3.8 (0) |
| 7                 | 6.8    |     | 0.5               | ---    | 6.8 (0) |
| 10                | 0.8    | 8.8  | 1.0               | 1.8 (0) | 8.8 (0) |
| 15                | 3.8    |     | 2.0               | 1.8 (0) | 8.8 (0) |
| 20                | 5.8    |     | 3.0               | 6.8 (0) | 8.0 (0) |
| 30                | 6.8    |     | ---               | ---    | ---   |

| ED50 (mg/kg) | 18.0 | 4.4 |
| (95% confidence limits) | (17.1–18.9) | (2.5–7.5) |

**Fig. 1.** Changes in anti-MES convulsant effects of diazepam, phenylacetylure and dipropylacetic acid (DPA) after olfactory bullectomy in mice.
FIG. 2. Changes in anti-pentetrazol convulsant effects of diazepam, phenylacetylurea and dipropylacetic acid (DPA) after olfactory bulbectomy in mice.

| MET (mA) | saline intact | saline O.B. | diazepam intact | diazepam O.B. |
|----------|---------------|-------------|----------------|---------------|
| 6        | 1/8           | --          | --             | --            |
| 7        | 4/8           | --          | --             | --            |
| 8        | 6/8           | 1/8         | 1/8            | --            |
| 10       | --            | --          | 3/8            | --            |
| 12       | --            | 3/8         | 5/8            | --            |
| 14       | --            | 4/8         | --             | --            |
| 15       | --            | 6/8         | --             | 0/8           |
| 17       | --            | --          | --             | 3/8           |
| 25       | --            | --          | --             | 5/8           |
| 50       | --            | --          | --             | 6/8           |

CD50 (mA) (95% confidence limits) 7.1 (5.6–8.6) 13.5 (10.8–16.9) 11.0 (9.2–13.1) 25.0 (22.3–27.9)

(5.6–8.6) mA for the intact mice and 13.5 (10.8–16.9) mA for the O.B. mice; i.e. the susceptibility of mice to electroshock convulsion was reduced by olfactory bulb ablations. After DZP was given to mice at a dose of 2 mg/kg s.c., greater electric current was required to induce convulsion, and this effect of DZP was also greater in the O.B. mice than in the intact animals.

Nitrazeepam (NZP): MES convulsion was tested 50 min after p.o. administration of NZP. NZP was also more effective in the O.B. mice than in the intact animals for preventing MES convulsion.

Phenobarbital (PHB)

MES convulsion was tested 90 min after and pentetrazol was injected 80 min after s.c. administration of PHB. No significant difference was found in the anticonvulsant effect of PHB between the O.B. and intact mice on both MES and pentetrazol convulsions, as can be seen in Table 4.

Diphenylhydantoin (DPH)

MES test was performed 120 min after s.c. administration of DPH. The anticonvulsant
TABLE 4. Comparison of anticonvulsant effects of phenobarbital in intact and O.B. mice

| dose (mg/kg s.c.) | MES convulsion | pentetrazol convulsion |
|------------------|----------------|------------------------|
|                  | intact         | O.B.                   | intact         | O.B.               |
| 15               | 2/8            | 2/8                    | 3/8 (0)        | —                  |
| 20               | 4/8            | 4/8                    | 5/8 (0)        | 2/8 (0)            |
| 30               | 7/8            | 6/8                    | 6/8 (0)        | 4/8 (0)            |
| 40               | —              | —                      | —              | 6/8 (0)            |
| ED50 (mg/kg)     | 25.0           | 29.0                   | 22.0           | 26.6               |
| (95% confidence limits) | (19.8–31.2) | (23.2–35.1)             | (18.0–26.1)    | (18.1–35.1)        |

effect of DPH in the O.B. mice was almost the same as in the intact animals.

When pentetrazol 85 mg/kg was injected s.c. 110 min after s.c. administration of DPH, greatly exaggerated convulsions were induced, as compared with that in the saline group, even in the intact mice; i.e. severe clonic convulsions frequently occurred and 2 out of 8 mice died in the group of DPH 50 mg/kg. This tendency was more marked in the O.B. mice than in the intact animals and 4 out of 8 O.B. mice died after pentetrazol administration in the DPH 50 mg/kg group.

Trimethadion (TMD)

The effect of TMD measured 90 min after s.c. administration of TMD, was almost the same in both the intact and O.B. mice.

Pentetrazol was injected s.c. 80 min after s.c. administration of TMD. No significant difference was found in the ED50 for pentetrazol convulsion between the intact and O.B. mice.

Acetazolamide (AZA)

MES convulsion was examined 180 min after s.c. administration of AZA. AZA exerted anticonvulsant effects at doses over 200 mg/kg s.c. in the intact mice, while effects were seen even at a dose of 100 mg/kg s.c. in the O.B. mice (Table 5). The ED50 of AZA in the O.B. mice was significantly smaller than that in the intact mice.

When pentetrazol was injected 170 min after s.c. administration of AZA, no change was observed in pentetrazol convulsion even at a dose of AZA 500 mg/kg s.c. both in the

TABLE 5. Comparison of anticonvulsant effects of acetazolamide in intact and O.B. mice

| dose (mg/kg s.c.) | MES convulsion |
|------------------|----------------|
|                  | intact         | O.B. |
| 100              | 0/8            | 1/8  |
| 200              | 2/8            | 5/8  |
| 300              | 3/8            | 6/8  |
| 400              | 7/8            | —    |
| ED50 (mg/kg)     | 285.0          | 200.0|
| (95% confidence limits) | (274.0–296.4) | (194.7–206.0)|
intact and O.B. mice.

*Phenylacetylene (PAU)*

The effect of PAU on MES convulsion was measured 45 min after p.o. administration of PAU. The results are shown in Fig. 1. The ED50 of PAU was significantly larger in the O.B. mice than that in the intact animals, indicating that the O.B. mice are less susceptible to the effect of PAU.

When pentetrazol was injected s.c. 35 min after p.o. administration of PAU, the anticonvulsant ED50 of PAU was 121.0 mg/kg in the intact mice, but PAU was ineffective in the O.B. mice even at a dose of 200 mg/kg. The dose of PAU was therefore increased to 300 mg/kg s.c. in the O.B. mice in order to obtain the ED50 (Fig. 2). The O.B. mice were considerably less susceptible to the effect of PAU.

*Dipropylacetic acid (DPA)*

MES convulsion was measured 25 min after s.c. administration of DPA. DPA protected TE of MES convulsion at doses over 250 mg/kg in the intact mice, but was ineffective even at a dose of 500 mg/kg in the O.B. mice (Table 6 and Fig. 1).

Pentetrazol was injected 15 min after s.c. administration of DPA. The anti-pentetrazol ED50 of DPA was 86.0 mg/kg s.c. in the intact mice, but doses over 150 mg/kg were required to protect against pentetrazol convulsion in the O.B. mice (Table 6 and Fig. 2).

The ED50 of all drugs tested for both the MES and pentetrazol convulsions in the intact and O.B. mice are summarized in Table 7.

**DISCUSSION**

In the present experiments, it was found that the sensitivity of mice to anticonvulsant drugs changed after bilateral ablations of the olfactory bulb, with concomitant appearance of hyperemotionality. From these changes, the anticonvulsant drugs can be categorized into 3 groups as follows: (1) drugs with increased sensitivity (benzodiazepines, AZA), (2) drugs with reduced sensitivity (PAU, DPA), (3) drugs without sensitivity changes (PHB, DPH, TMD). The increase in sensitivity to benzodiazepines was the most significant after olfactory bulbectomy in both MES and pentetrazol convulsions.

Five possible explanations are proposed for altered sensitivity to drugs after olfactory
| Drug                  | Anti-MES convulsion | Anti-pentetrazol convulsion |
|----------------------|---------------------|-----------------------------|
|                      | intact mice | O.B. mice | ratio | intact mice | O.B. mice | ratio |
| Chloridiazepoxide (s.c.) | 96.8 (79.2–114.4) | 27.4 (14.1–37.8) | 3.53* | 7.6 (5.6–9.2) | 3.6 (2.2–5.1) | 2.11* |
| Diazepam (s.c.)     | 18.0 (17.1–18.9) | 4.4 (2.5–7.5) | 4.1* | 1.9 (1.1–2.8) | 0.3 (0.2–0.5) | 6.33* |
| Nitrazepam (p.o.)   | 72.0 (68.4–77.8) | 31.3 (22.6–39.9) | 2.30* |  |  |  |
| Phenobarbital (s.c.)| 25.0 (19.8–31.2) | 29.0 (23.2–35.1) | 0.86 | 22.0 (18.0–26.1) | 26.6 (18.1–35.1) | 0.83 |
| Diphenylhydantoin (s.c.) | 14.8 (11.1–18.8) | 12.8 (8.7–16.9) | 1.16 | 50.0 | 50.0 | --- |
| Trimethadion (s.c.)  | 660.0 (616.3–705.2) | 640.0 (603.7–677.4) | 1.03 | 490.0 (457.8–523.3) | 540.0 (503.7–577.8) | 0.90 |
| Acetazolamide (s.c.) | 285.0 (274.0–296.4) | 200.0 (194.7–206.0) | 1.43* | 500.0 | 500.0 | --- |
| Phenylacetylurea (p.o.) | 110.0 (82.4–138.5) | 165.0 (141.1–189.7) | 0.67* | 121.0 (99.2–143.1) | 236.0 (195.0–285.6) | 0.51* |
| Dipropylacetic acid (s.c.) | 310.0 (278.1–345.6) | >500.0 (71.7–103.2) | 0.62* | 86.0 (146.3–221.4) | 188.3 | 0.46* |

Statistical significance (p < 0.05) is indicated by asterisk.
bullectomy. The first is that alterations in the blood-brain barrier occur as the result of brain damage. It is reported that even in cortical damage, the permeability of blood-brain barrier increases for a few days and then returns to normal (3). In olfactory bulbectomy, the change would therefore be expected to be at a maximum a few days following the lesion. In this study, the experiments were started 2 weeks after lesions. Furthermore, changes in sensitivity to anticonvulsants after olfactory bulbectomy are not only increment but there was also reduction or no change, thus it is hardly conceivable that changes in sensitivity are due to alterations in the blood-brain barrier.

The second explanation is that changes in sensitivity to these drugs are correlated to reduced susceptibility to convulsions induced by electroshock or pentetrazol after olfactory bulbectomy. As the susceptibility to convulsions is reduced after olfactory bulbectomy, such could account for increased sensitivity to anticonvulsants such as benzodiazepines following lesions. However, the increment of sensitivity to benzodiazepines was marked; e.g. the anticonvulsant potency of diazepam in O.B. mice is about 6 times that in intact mice in the pentetrazol convulsion (1). It is therefore unlikely that the results are due to reduced susceptibility to convulsions.

The third explanation is that the lesions infringed on specific receptor sites for the drug action. Because the anticonvulsant effect of dipropylacetic acid was completely abolished after olfactory bulbectomy, it is possible that the site of action of dipropylacetic acid exists in either the olfactory bulb itself or in areas directly connected.

The fourth explanation proposed is that the lesion infringed on an area remote from the site of action of the drug, which normally regulates the overall effect of the drugs. There is evidence that one of the sites of action of benzodiazepines is the amygdaloid complex where the olfactory afferent fibers terminate through the lateral olfactory tract and the activity appears to be influenced by olfactory bulbectomy as a result of either the absence of olfactory impulses or degeneration of the olfactory afferent fibers (4, 5). In fact, Callens et al. (6) reported that the excitability of the prepyriform cortex increased after removal of the olfactory bulb and changes in the amygdaloid activity were also evidenced by the recent finding in our laboratory that the voltage of EEG activity in the amygdala was much reduced after olfactory bulbectomy in rats with chronic electrode implants (7). Removal of these regulatory influences on the amygdaloid complex could then account for the increased responsiveness to benzodiazepines following olfactory bulbectomy. Except for the amygdaloid complex, the afferent fibers from the olfactory bulb distribute to the anterior olfactory nuclei, olfactory tubercle, prepyriform and pyriform cortices. The activities of these limbic areas connected with the olfactory bulb may be changed after olfactory bulbectomy. Although the role of the limbic system in convulsions remains unknown and the sites of action of anticonvulsants remain to be elucidated, the altered activity in these limbic areas may account for the increased or decreased sensitivity to anticonvulsant drugs such as benzodiazepines, acetazolamide, phenylacetylurea and dipropylacetic acid in both MES and pentetrazol convulsions.

In the O.B. mice, some areas of the anterior olfactory nuclei were also lesioned in this
experiment. The fibers from these nuclei connect with the hypothalamus and midbrain through the medial forebrain bundle. The activity of the hypothalamus and the midbrain may also be influenced directly by olfactory bulbectomy, and indirectly with altered activity of the other limbic structures such as the amygdala, piriform cortex, olfactory tubercle etc. The O.B. mice are maintained at a higher arousal level, indicating increased activity of the midbrain reticular activating system. Recently, it was found that removal of the olfactory bulb increased norepinephrine levels with elevated activity of monoamine oxidase (MAO) in the brainstem whereas the removal decreased norepinephrine with lowered MAO activity in the telencephalon (8). There is evidence that one of the sites of action of benzodiazepines is the midbrain reticular formation which plays an important role in the development of tonic convulsion in the MES (9). Therefore, the increased activity of the midbrain reticular activating system may account for the increased sensitivity to benzodiazepines in O.B. mice.

As the sensitivities of phenobarbital and trimethadion were not altered by olfactory bulbectomy in both MES and pentetrazol convulsions, the site and mechanism of action of these drugs may be different from those of other drugs.

The fifth explanation is that denervation supersensitivity occurred as the result of olfactory bulbectomy. If the denervation supersensitivity is involved, the time of onset of the change in drug sensitivity must be gradual. In this experiment, we did not study whether the changes in sensitivity to anticonvulsants occurred immediately or gradually after olfactory bulbectomy. However, the hyperemotionality appeared gradually and reached a maximum 1 to 2 weeks after olfactory bulbectomy. This phenomenon suggested that hyperemotionality induced by olfactory bulbectomy is partially due to denervation supersensitivity of the areas connected with the olfactory bulb or anterior olfactory nuclei, such as amygdala, olfactory tubercle, hypothalamus, midbrain etc. Therefore, the denervation supersensitivity of these areas could account for the increased sensitivity to anticonvulsant drugs such as benzodiazepines and acetazolamide following olfactory bulbectomy.

In general, of the mechanisms considered here, the fourth and fifth explanations appear to be the most relevant for the changes in sensitivity to anticonvulsant drugs following olfactory bulbectomy.

Further biochemical and physiological investigations are under way to elucidate the mechanism of increased or decreased sensitivity to anticonvulsants after olfactory bulbectomy.

Acknowledgements: This investigation was supported in part by a grant from the Ministry of Education, Japan and from the Takeda Science Foundation.

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