binding domain of the 2C6 molecule and studies are in progress to determine whether or not these antiidiotypic antibodies can recognize the BZD receptor complex.

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REFERENCES

1. W. Haefely, *J. Psychoact. Drugs* **15**, 19 (1983).
2. C. Braestrup and M. Nielsen, in *Handbook of Psychopharmacology*, Vol. 17 (Eds. L. L. Iversen, S. D. Iversen and S. H. Snyder), p. 285. Plenum Press, New York (1983).
3. H. Möhler and J. G. Richards, in *Anxiolytics: Neurochemical Behavioural and Clinical Perspectives* (Eds. J. B. Malick, S. J. Enna and H. I. Yamamura), p. 15, Raven Press, New York (1983).
4. H. Möhler, M. Battersby and J. Richards, *Proc. natn. Acad. Sci. U.S.A.* **77**, 1666 (1980).
5. H. Möhler and T. Okada, *Science* **198**, 849 (1977).
6. T. Taniguchi, J. K. T. Wang and S. Spector, *Biochem. Pharmac.* **31**, 589 (1982).
7. R. F. Squires and C. Braestrup, *Nature, Lond.* **266**, 732 (1977).
8. J. W. Regan, H. I. Yamamura, S. Yamada and W. R. Roeske, *Life Sci.* **18**, 991 (1981).
9. L. Sangameswaran and A. L. de Blas, *Proc. natn. Acad. Sci. U.S.A.* **82**, 5560 (1985).
10. A. L. de Blas, L. Sangameswaran, S. A. Haney, D. Park, C. J. Abraham, Jr. and C. A. Rayner, *J. Neurochem.* **45**, 1748 (1985).
11. R. Dixon in *Methods in Enzymology*, Vol. 84 (Eds. J. J. Langone and H. Van Vunaks), p. 490. Academic Press, New York (1982).
12. P. Parham, M. Androlewicz, F. M. Brodsky, N. J. Holmes and J. P. Ways, *J. Immun. Methods*, **53**, 133 (1982).
13. C. Braestrup and R. Squires, *Proc. natn. Acad. Sci. U.S.A.* **74**, 3805 (1977).

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Effects of carbon dioxide on onsets of seizures in mice induced by antagonists of vitamin B_6

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The excitability of the central nervous system is affected considerably by procedures that change the carbon dioxide concentration in blood and tissues. For example, the increased blood concentration of CO_2 induced by the inhalation of CO_2 (5–20%) increases the threshold for minimal electroshock seizures [1], whereas the fall in arterial pCO_2 induced by hyperventilation increases electrical activity of the brain cortex [2]. Further, it is well known clinically that petit mal seizures can be induced by hyperventilation and abated by the addition of moderate amounts of CO_2 to the inspired air [3, 4].

In investigating the possible mechanism of the action of CO_2, it was deemed important to study the effect of the gas on experimental seizures, the mechanism of which has been, comparatively, made clear. Antivitamin B_6 is known to produce in experimental animals characteristic convulsions that are related to a decline in γ-aminobutyric acid (GABA, an inhibitory transmitter) because of an inhibition of glutamic acid decarboxylase in the central nervous system [5]. And it is assumed that a decrease in release of GABA from the nerve terminals and, thereby, a decline in binding of GABA to its receptor of the postsynaptic neuron produce an enhancement in central neuronal excitability [6]. In this paper, we have investigated the effect of inhalation of CO_2 on seizures induced by injection of DL-penicillamine (PeA) and thiosemicarbazide (TSC) which are known to be antagonists of vitamin B_6.

Methods

Exposure of animals to carbon dioxide. DDY mice weighing 20–25 g were used as experimental animals. The animals were exposed to various gas mixtures by placing them in individual plastic chambers (6 cm high × 12 cm deep × 6 cm long) with the following details of construction. A 3-cm circular opening was made on one side to introduce the animal into the chamber, and the opening was closed by a rubber stopper that was pierced with two glass-tubes (3 mm d) for entrance of the gas mixture into the chamber and exit of it from there. The tube that was the entrance to one chamber was connected to the tube that was the exit of the adjacent chamber, and the exit tube was connected to the entrance tube of another adjacent chamber.

The gas mixture to be delivered to the chain of chambers was prepared by mixing the gases in a large gasometer (24 liter bottle). The various gases, obtained from separate tanks of oxygen, nitrogen and carbon dioxide, were mixed in the gasometer in the proper proportions. In this manner, any mixture of gases desired for a particular experiment could be obtained; all mixtures contained 20% oxygen plus nitrogen as the diluent. By pouring water into the gasometer the gas mixture was expelled into all the chambers through the entrance tube of the first chamber.

After the air in the chambers had been replaced with a gas mixture to be tested, the animals were introduced through the openings into the respective chambers, and then the gas mixture flow through the animal chambers was maintained at a constant rate. The rate of flow of the gas was 2 liters per 10 min. The behavior of the animals was observed continuously through the plastic walls of the chambers.

Induction of convulsion by convulsants. PeA and TSC were used as convulsants. Solutions of these drugs were prepared daily in 0.9% NaCl solution, the pH being adjusted to 7 immediately before use. The final concentration of the drugs was adjusted so that the required dose was administered in a volume equivalent to 1% of the body weight of the animal. All injections were intraperitoneal, and the injected animals were kept in their usual cage until they were introduced into their chambers. After the injections, food and water were withheld from the animals during experiments.

Results

The exposure of mice to a 5% CO_2 gas mixture, 100 min after the administration of PeA (2 mmol/kg), had little effect on the total number of animals convulsing but had a

significant effect in extending by about 10 min the time to onset of convulsions (Fig. 1a). Exposure to 10% CO_2 appreciably protected mice from the PeA-induced convulsions, and the action of CO_2 was evident both by the decreased percentage of mice convulsions and by the increase in the time to onset of convulsions; the protective action observed on the incidence of convulsions was continued even after a change from CO_2 to air (Fig. 1b). Exposure to 20% CO_2 protected mice appreciably more from the convulsions. No PeA-induced seizures occurred during the exposure, and even after the change to air, only one mouse out of twenty convulsed at 90 min after the change; the remaining nineteen mice were spared completely from convulsion (Fig. 1c).

Next, the effect of CO_2 on TSC-induced convulsions was studied. The exposure of mice injected with TSC (0.2 mmol/kg) to 10% CO_2 protected mice appreciably from the TSC-induced convulsions; the protective action was evident by the decrease in the incidence of convulsions and by the increase in the latent time (Fig. 2a). Mice exposed to 20% CO_2 were protected appreciably more from the convulsions. As shown in Fig. 2b, no seizures occurred during the CO_2 exposure. In contrast to the experiments with PeA, however, the exposure to CO_2 did not prevent the onset of convulsions after the change to air, and all animals had convulsions in rapid succession in the course of a few minutes.

Discussion

As described at the beginning of the paper, vitamin B_6 antagonists produce in experimental animals the characteristic convulsions that are considered to be related to inhibition of glutamic acid decarboxylase and to a resulting decline in binding of GABA to its receptors [6, 7].

The effects of inhalation of carbon dioxide on the vitamin B_6 antagonist-induced convulsions are shown in Figs. 1 and 2. Concentrations of 10–20% carbon dioxide were able to protect mice against the seizures induced by both PeA

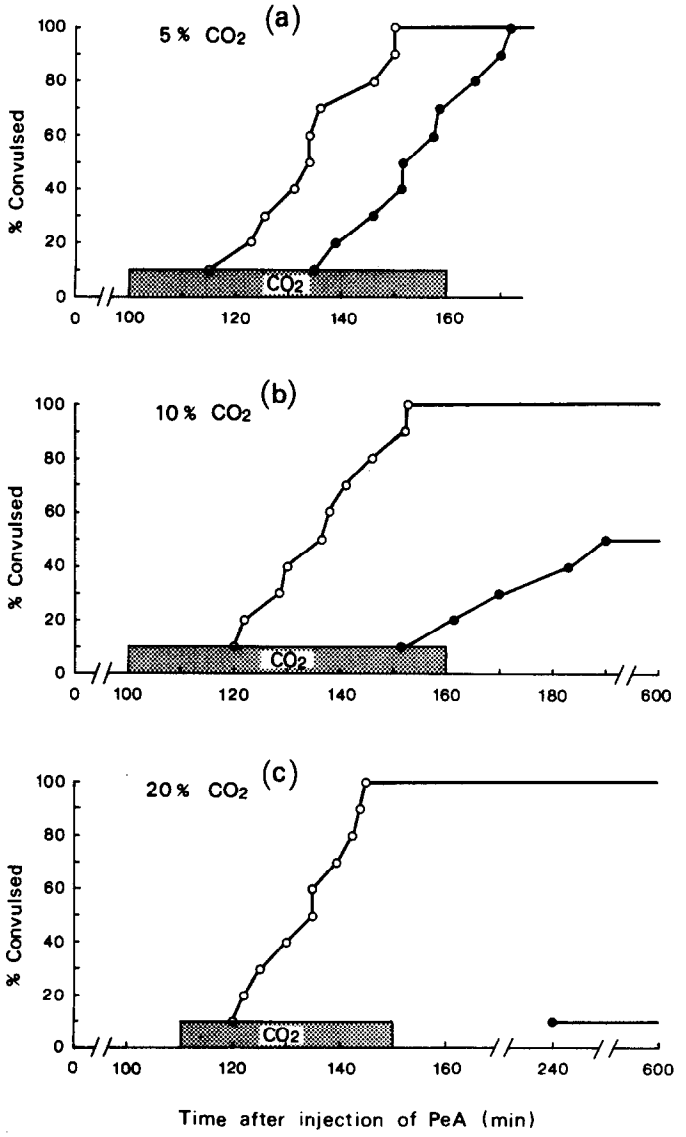

Fig. 1. Effect of inhalation of CO_2 on the incidence of seizures induced in mice by injection of PeA (2 mmol/kg). The period of CO_2 exposure is indicated by a shaded rectangle. Key: (○—○) control (room air); and (●—●) CO_2 exposure.

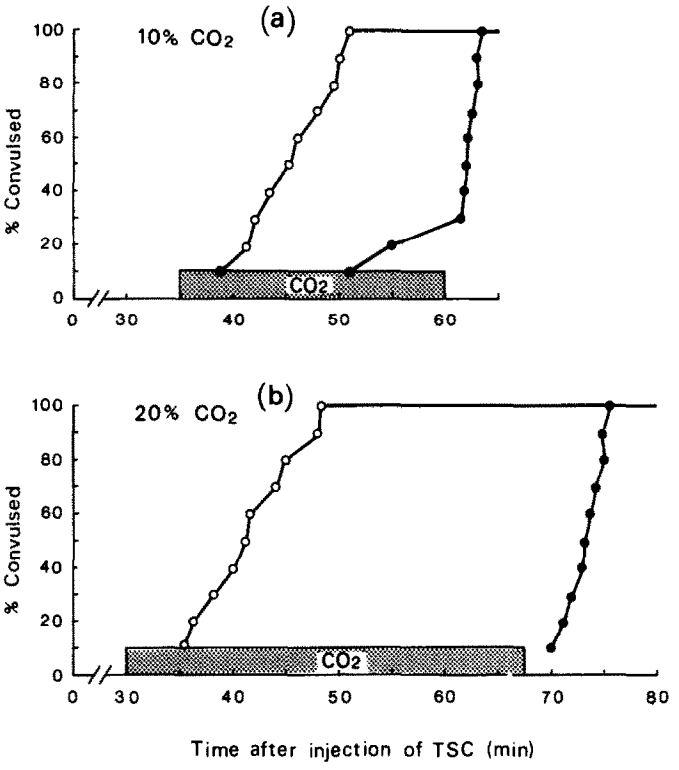

Fig. 2. Effect of inhalation of CO_2 on the incidence of seizures induced in mice by injection of TSC (0.2 mmol/kg). The period of CO_2 exposure is indicated by a shaded rectangle. Key: (○—○) control (room air); and (●—●) CO_2 exposure.

and TSC, used as antagonists of vitamin B_6, though the protective effect on the PeA-induced seizures tended to continue after the return of the animals to normal air.

When investigating the possible mechanisms of this protective action on the vitamin B_6 antagonist-induced convulsions, we must consider all the diverse actions of the gas on many tissues. Breathing an atmosphere with increased CO_2 may well raise the intracellular CO_2 concentration [8] and consequently, lower the intracellular pH in brain and, thus, decrease the excitability of neuronal cells. For example, the pH optimum of glutamic acid decarboxylase is on the acid side of normal pH [9, 10], and so the enzyme activity may be enhanced and GABA formation may be increased as a result of CO_2 inhalation. The increase of CO_2 concentration in brain also may decrease the excitability of neuronal cells through GABA receptors, whose *in vitro* affinity for GABA has been shown recently to be enhanced in the presence of increased CO_2 [11, 12]. On the other hand, the exposure to increased CO_2 also could result in the liberation of hormones, such as adrenocortical hormones, which are known to alter excitability of neuronal tissues [13].

This situation, however, is probably much more complex than is described above. The mechanism of the anticonvulsant effect of CO_2 requires much further investigation not only on the GABA system but also on many other possibilities.

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REFERENCES

1. D. M. Woodbury, L. T. Rollins, G. G. Richards and P. S. Timiras, *Fedn Proc.* **14**, 395 (1955).
2. J. E. P. Toman and J. P. Davis, *Pharmac. Rev.* **1**, 425 (1949).
3. W. G. Lennox and F. A. Gibbs, *Archs Neurol. Psychiat.* **36**, 1236 (1936).
4. J. S. Meyer, F. Gotoh and E. Favele, *Electroenceph. clin. Neurophysiol.* **21**, 10 (1966).
5. K. F. Killam and J. A. Bain, *J. Pharmac. exp. Ther.* **119**, 255 (1957).
6. M. Matsuda, M. Abe, M. Hoshino and T. Sakurai, *Biochem. Pharmac.* **28**, 2785 (1979).
7. M. Abe, M. Hoshino and M. Matsuda, *Jikeikai med. J.* **29**, 233 (1982).
8. D. A. Brodie and D. M. Woodbury, *Am. J. Physiol.* **192**, 91 (1958).
9. J-Y. Wu, T. Matsuda and E. Roberts, *J. biol. Chem.* **248**, 3029 (1973).
10. J-M. Blindermann, M. Maitre, L. Ossola and P. Mandel, *Eur. J. Biochem.* **86**, 143 (1978).
11. S. Kurioka, Y. Kimura and M. Matsuda, *J. Neurochem.* **37**, 418 (1981).
12. S. Hori, S. Kurioka and M. Matsuda, *Jikeikai med. J.* **30**, 429 (1983).
13. D. M. Woodbury, *Pharmac. Rev.* **10**, 275 (1958).