ACTION SITE OF ANTAGONISTS OF VITAMIN B₆ IN THE CENTRAL NERVOUS SYSTEM OF THE FROGS AND COCKROACHES¹

Junko YAMASHITA²

Department of Biochemistry, Jikei University School of Medicine, Tokyo 105, Japan

(Received January 10, 1975)

Summary Susceptibility to some antagonists of vitamin B₆ was studied in frogs and cockroaches after operational intervention in their central nervous systems, and also in the intact frogs and cockroaches.

Thiosemicarbazide, semicarbazide, isoniazide and penicillamine induced wild jumping behavior, and tonic or clonic convulsions in frogs, when the nervous parts posterior to the optic lobe inclusive remained intact. In frog, in which the nervous parts anterior to the diencephalon inclusive had been removed, no convulsions were induced by castrix or 4-deoxy-pyridoxine in large doses. In cockroaches excessive fluttering of wings and convulsions upon administration of thiosemicarbazide following severance of the central nerve cord between the subesophageal and prothoracic ganglions were induced. Castrix was not a convulsant in the intact frogs and cockroaches.

Data presented in the previous paper have shown that administration of thiosemicarbazide (TSC), an antagonist of vitamin B₆ (B₆), induced restlessness, active behavior and convulsions in frogs and cockroaches (7). We know that frogs or cockroaches are able to survive for a long time after such a severe operation as decapitation or severance of the central nerve, and that such animals are used in the study of the site of action of the drugs. The present paper reports on some antagonists of B₆ induced active behavior and convulsions in frogs and cockroaches.

MATERIALS AND METHODS

TSC (Wako Chemicals), semicarbazide hydrochloride (SC, Wako Chemicals), isoniazide (INH, Tokyo Kasei Chemicals), castrix (Takeda Chemicals), DL-penicil-
lamine (Nakarai Chemicals), 4-deoxypyridoxine hydrochloride (DOP, Nakarai Chemicals) and toxopyrimidine (TXP, Takeda Chemicals) were used as antagonists of $B_6$. SC and DOP were neutralized with NaOH before using. Experimental animals were treated after the method described previously (1).

In autumn, frogs (Rana nigromaculata) underwent the following operations. In the serise of experiments on spinal frogs, the nervous part between the medulla and spinal cord was transsectioned with a small knife 3 hr after TSC administration. In the experiments on decerebrated frogs, the nervous parts anterior to the cerebrum inclusive were removed with the upper jaw by the aid of scissors, and immediately a $B_6$ antagonist was given. In some decerebrated frogs, the diencephalon and optic lobe were further removed with a knife after the skull had been cut further, and they were then treated just as the decerebrated frogs. All the operated frogs were placed in 5-liter beakers with water (about 1 cm in depth), and observed 6 hr after drug administration.

In cockroaches (Periplaneta americana) anesthetized with ethyl ether, the central nerve cord between the subesophageal and prothoracic ganglions was severed with two forceps under a binocular microscope. The insects were given TSC 4 hr after operation, and observed for 5 hr.

RESULTS

Effect of TSC after an operation on the central nervous system of frog

In frogs upon which operations of the spinal cord and the nervous parts anterior to the optic lobe had been performed no active locomotion nor convulsions after treatment with TSC were shown. Susceptibility to 3 $\mu$g/g of strychnine

| Operation                                                     | Number convulsing/treated | Latent period of the first convolution min (±S.D.) | Number dead/treated |
|---------------------------------------------------------------|---------------------------|---------------------------------------------------|---------------------|
| Transsection between the medulla and spinal cord (spinal frog) | 0/5                       | --                                                | 0/5                 |
| Removal of the nervous parts anterior to the optic lobe inclusive | 0/5                       | --                                                | 0/5                 |
| Removal of the nervous parts anterior to the diencephalon inclusive | 3/3                       | 202±46                                            | 0/3                 |
| Removal of the nervous parts anterior to the cerebrum inclusive (decerebrated frog) | 6/6                       | 156±61                                            | 3/6                 |
| Unoperated                                                    | 6/6                       | 264±64                                            | 1/6                 |

*a The statistical significance in comparison with the group not operated upon was determined by $t$-test.
sulfate on the spinal frogs was confirmed as the technical control. In the decerebrated frog or the frog with the nervous parts anterior to the diencephalon inclusive removed wild leaping or jumping behavior, and tonic or clonic convulsions about 2.5 hr after TSC treatment were induced. In the decerebrated frogs, the latent period of the first convulsion was shorter than that in frogs that did not undergo operation (Table 1).

Effect of other antagonists of B6 on the frog with the nervous parts anterior to the diencephalon inclusive removed

SC (1,200 μg/g), INH (900 μg/g), DL-penicillamine (800 μg/g) and TXP (1,000 μg/g) were found to induce wild leaping or jumping behavior, and tonic or clonic convulsions in the frog in which the nervous parts anterior to the diencephalon inclusive had been removed. The latent period of the first convulsion was about 3 hr. Such an anomaly of behavior was not observed in the frogs given DOP and castrix at the doses of 6–10 (2) and 30–80 (3) times, respectively, of LD50 in mice (Table 2).

Table 2. Effect of intraperitontial administration of B6 antagonists on frog with the nervous parts anterior to the diencephalone inclusive removed.

| B6 Antagonists | Dose μg/g | Number convulsing | Number treated | Latent period of the first convulsion (±S.D.) | Number dead | Number treated |
|----------------|-----------|-------------------|---------------|--------------------------------|-------------|---------------|
| SC             | 1,200     | 3/4               |               | 180±60 | 2/4          |              |
| INH            | 900       | 2/3               |               | 117,233 | 1/3          |              |
| DL-penicillamine| 800       | 2/4               |               | 261,267 | 0/4          |              |
| TXP            | 1,000     | 3/3               |               | 176±117 | 0/3          |              |
| DOP            | 600       | 0/2               |               | —       | 0/2          |              |
|                | 1,000     | 0/1               |               | —       | 0/1          |              |
|                | 1,500     | 0/2               |               | —       | 0/2          |              |
| Castrix        | 40        | 0/3               |               | —       | 0/3          |              |
|                | 100       | 0/4               |               | —       | 0/4          |              |

Table 3. Effect of castrix on intact frogs and cockroaches, and of thiosemicarbazide on cockroaches with severed nerve cord.

| Animals     | Operation | B6 Antagonists | Dose μg/g | Number convulsing | Number treated | Latent period of the first convulsion (±S.D.) |
|-------------|-----------|----------------|-----------|-------------------|---------------|---------------------------------------------|
| Frog        | Intact    | Castrix        | 100       | 0/3               |               | —                                           |
| Cockroach   | Intact    | Castrix        | 0.2       | 0/5               |               | —                                           |
|             |           |                | 2         | 0/5               |               | —                                           |
|             |           |                | 40        | 0/5               |               | —                                           |
|             |           |                | 100       | 0/5               |               | —                                           |
| Cockroach   | with severed nerve cord | TSC | 300       | 5/5               | 128±39         | —                                           |

* The central nerve cord was severed between the subesophageal and prothoracic ganglions.
Effect of TSC on cockroach with a severed central nerve cord

After severance of the central nerve cord between the subesophageal and prothoracic gangliaons, convulsions were induced in cockroach about 2 hr after administration of 300 μg/g of TSC (Table 3).

Effect of castrix on frog and cockroach not operated upon

In frogs not operated upon, neither active locomotion nor convulsions were induced by 100 μg/g of castrix. Cockroaches, treated with 0.2, 2, 40 or 100 μg/g of castrix, showed such abnormal behavior as laterally turning their bodies and raising their limbs. But no convulsion was induced (Table 3).

DISCUSSION

Treatment with TSC, SC, INH, penicillamine or TXP induced wild leaping or jumping behavior, and tonic or clonic convulsions in the frog in which the nervous parts posterior to the optic lobe inclusive remained intact. Convulsions were induced in the frog treated with the same dose of TSC as that of the frogs not operated upon. Consequently, the olfactory lobe, cerebrum and diencephalon were found not to be stimulated mainly by the B₆ antagonist. On the contrary, neither abnormal behavior nor convulsions were induced by TSC in frog upon which an operation on the spinal cord was performed as well as in those in which the nervous parts anterior to the optic lobe inclusive were removed. MINESHITA et al. (4) reported that TXP is not a convulsant in decapitated frogs. This datum and the present finding suggest that the B₆ antagonists have a most selective effect on the optic lobe. Provoking of TSC convulsions may be inhibited by the action of the cerebrum, because the latent period of the first convolution in decerebrated frogs was observed to be shorter than that in frogs not operated upon.

Excessive restlessness and convulsions were found in the cockroaches treated with TSC after severance of the central nerve cord between the subesophageal and prothoracic ganglion. The brain appears to be dispensable for inducing TSC convulsions in the cockroach. Concerning the cockroach, there are many reports that decapitation or the severance of the central nerve cord causes no decrease of susceptibility to the nerve-poisoning insecticides, e.g. DDT (1-trichloro-2,2-bis(p-chlorophenyl)-ethan) (5). B₆ antagonists seem to have a convulsant action on the same site of the nervous system as the insecticides.

Since animals other than mammals showed higher threshold of convulsions to TSC (1), the site of action of B₆ antagonists on the nervous system may differ among animals. However, the fundamental mode of action of B₆ antagonist on provoking of convulsions is considered to be similar. The frog or cockroach, whose nervous system presents a more primitive state of evolutionary development than that of the mouse or rat, is expected to be better experimental material in a search for the fundamental mechanism of the convulsions.
The author is grateful to Dr. M. Matsuda for his helpful discussion and Dr. C. Isojima for his technical advice.

REFERENCES

1) YAMASHITA, J., *J. Nutr. Sci. Vitaminol.*, 20, 113 (1974).
2) KARLOG, O. and KNUDSEN, E., *Nature*, 200, 790 (1963).
3) NOZAKI, J., *Vitamins*, 14, 310 (1958).
4) MINESHITA, T., KIDO, R., OGAWA, Y., TAKAHASHI, S., TANAKA, A., HIRAOKA, K., HIROSE, K., ISHIGAMI, T., KUROZAWA, J., SEKIHARA, S., and HIRIHE, K., *Ann. Rept. Shionogi Res. Lab.*, 7, 575 (1957).
5) ROEDER, K. D. and WeiANT, E. A., *Science*, 103, 304 (1946).