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STUDIES ON 6-AZAURIDINE AND 6-AZACYTIDINE—IV.
CORRELATIONS BETWEEN METABOLISM AND CENTRAL NERVOUS EFFECTS OF 6-AZACYTIDINE

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Abstract—Effects of 6-azacytidine on exploratory activity were studied in mice and rats. After 200 to 800 mg of 6-azacytidine per kilogram were injected intraperitoneally, the exploratory activity of rats remained practically unaffected, whereas in mice this activity was depressed in a manner proportional to dosage. The lethal effects of nicotine also were antagonized in the mouse when higher doses of the compound (2·5 to 5·0 g/kg) were administered intraperitoneally. Neither changes in behavior nor antagonism to nicotine were observed in the same species after the injection of 6-azacytidine into the cerebral ventricles. In the cat, however, 50 to 100 mg of 6-azacytidine per kilogram, administered into the lateral cerebral ventricles, after a period of latency, produced ataxia and finally loss of postural reflexes.

The possible role of deamination of 6-azacytidine, with the formation of 6-azauridine, in the production of the central effects of 6-azacytidine in different animal species is discussed.

NEUROTOXICITY is an undesirable side-effect caused by some 6-azapyrimidines with cytostatic activity, especially 6-azauracil. Since the causal relationship between cytostatic activity and neurotoxic effects was questioned,[1] new evidence has accumulated[2, 3] to suggest that both effects might be based on the same primary mechanism, namely inhibition of orotidylic acid decarboxylase through the 6-azauridine-5′-monophosphate formed. It has been reported recently that 6-azacytidine, another cytostatic active pyrimidine analogue, investigated by Šorm, Smrt and Černěckij,[4] also is able to inhibit this enzyme to some extent, and by a similar mechanism.[5] Moreover, a partial deamination of 6-azacytidine to 6-azauridine in tumor-bearing mice has been described by Handschumacher, Škoda and Šorm.[5] The question arose, therefore, whether the administration of 6-azacytidine also might provoke alterations in some functions of the central nervous system, since in previous experiments with 6-azauridine[3] depressive effects on exploratory activity were detectable with relatively very low doses (1–2 per cent of the LD_{50}). In these experiments, 6-azauridine also appeared to be very active after direct administration into the cerebral ventricles of mice and cats.

METHODS

The experiments were performed on 180 white mice (strain H, stock Konárovice) of both sexes, weighing 18–22 g. The animals were divided into groups containing at least 10 animals. Also used for testing exploratory activity were 24 albino male rats of the Wistar strain, weight 180–220 g. Cats of both sexes, weighing 2·0–2·5 kg, were of various origin.

Exploratory activity was tested in mice and rats, according to the procedure of Lát,[6] slightly modified by us.[3] 6-Azacytidine was administered 90 min before the test and the percentage changes in exploratory activity under drug treatment were compared with corresponding changes in a parallel control group treated with the same volume of saline. The statistical evaluation of the differences was performed using the nonparametric U-test of Mann and Whitney.

The antagonism of the lethal effects of nicotine was evaluated by comparing the individual effective doses of nicotine producing death under pretreatment by 6-azacytidine and in saline-treated animals.[3] Nicotine bitatrate, as a 0·05 per cent (w/v) solution, was administered by slow intravenous infusion at a constant rate (3 ml/min) 2 hr after intraperitoneal and either 15 min or 2 hr after intraventricular injection of 6-azacytidine.

Injections into the lateral cerebral ventricles were performed in mice using the semiautomatic apparatus constructed by Krebs[7] and in 6 cats with implanted permanent cannulas, according to Feldberg and Sherwood.[8]

6-Azacytidine was dissolved in distilled water for intraperitoneal administration and in freshly prepared Tyrode's solution for injections into the cerebral ventricles. Constant volumes of the solutions—0·02 ml in mice and 0·5 ml in cats—were always injected intraventricularly.

RESULTS

The effects of 6-azacytidine on exploratory activity in mice and rats are summarized in Fig. 1. It appears that in the rat the exploratory activity remains practically unaffected by doses of 6-azacytidine up to 800 mg/kg given intraperitoneally. In the mouse, however, this activity begins to be depressed by 400 mg/kg and even more by 800 mg/kg of the compound, the effect of the latter dose being statistically significant ($p < 0·05$). In agreement with experiments using 6-azauridine, there were no parallel significant changes in activities irrelevant to the situation (grooming).

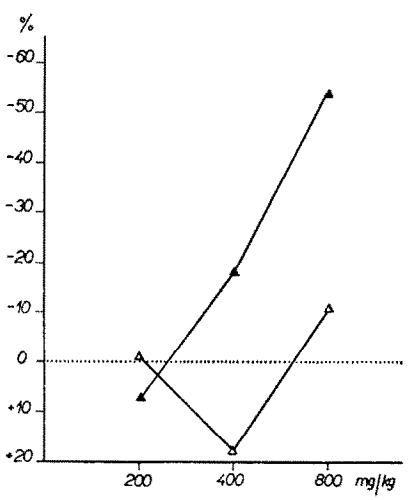

FIG. 1. Effect of 6-azacytidine on exploratory activity in mice ▲——▲ and rats △——△. The effects are expressed as the differences in the percentage changes in exploratory activity between the 6-azacytidine-treated group and control animals.

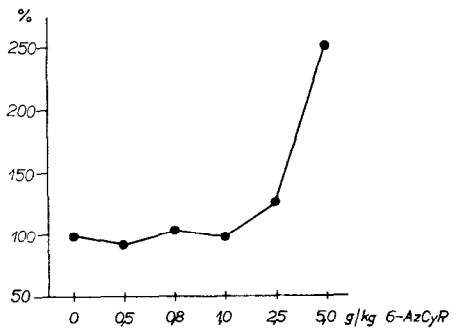

FIG. 2. Relative changes of mean individual lethal doses for nicotine after intraperitoneal administration of 6-azacytidine.

Figure 2 shows that in the mouse there is also a certain antagonism to the lethal effects of nicotine, since the individual lethal doses of this convulsant are raised significantly by 2·5–5·0 g of 6-azacytidine per kg injected intraperitoneally.

When administered directly into the lateral cerebral ventricles of mice, however, 6-azacytidine did not evoke any apparent changes in the behaviour of this animal species when given in doses up to 200 mg/kg intraventricularly. Simultaneously, we observed neither an incoordination on the rotating rod, nor a singificant increase in the amount of nicotine necessary to produce death, except a slight enhancement after 200 mg/kg when the infusion of nicotine was postponed for 2 hr after the administration of 6-azacytidine (Fig. 3).

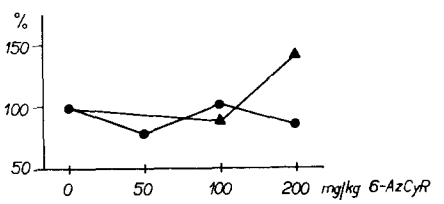

FIG. 3. Relative changes of mean individual lethal doses for nicotine after intraventricular injection of 6-azacytidine 15 min (●——●) and 2 hr (▲——▲) before nicotine infusion.

In cats, 6-azacytidine injected into the left lateral cerebral ventricles in the dose range 50–100 mg/kg, similarly did not evoke any particular behavioural pattern in the first period after its administration. Nevertheless, within 2 hr ataxia became a salient manifestation in 6-azacytidine-treated animals and this was followed by a progressive loss of postural reflexes. After 100 mg/kg the animals remained completely paralysed for more than 24 hr and all died within a few days. After 50 mg/kg, with which dose the complete paralysis of the animals was only transitory, all animals survived. In these animals, however, ataxia and sometimes paralysis of the hind-legs were of longer duration and the animals recovered only after several days.

DISCUSSION

In our experiments, 6-azacytidine was practically ineffective when tested on exploratory activity of rats in doses with which 6-azauridine significantly affects this type of behaviour.[3] On the other hand, clear cut effects have been observed in mice

when 6-azacytidine was administered intraperitoneally. The relative activity of 6-azacytidine varies between 25–35 per cent of that of the same doses of 6-azauridine. It should be considered, however, that in the mouse about one-third of administered 6-azacytidine is deaminated to 6-azauridine *in vivo*,[5, 9] whereas in the rat there is almost no deamination detectable.[9] The very weak deamination of 6-azacytidine in the rat, as well as the quantitative correlation between the rate of deamination and the intensity of observed effects in the mouse, strongly support the view that a great deal of central activity of 6-azacytidine in the mouse is a reflection of its deamination to 6-azauridine. The same interpretation holds true also for the discrepancy between the detected activity of 6-azacytidine after systemic administration and its apparent inactivity after intraventricular injection in the mouse, since no deamination has been found in the brain of all animal species hitherto studied.[9]

In contrast to mice, considerable effects were observed in cats after injection of 6-azacytidine into the cerebral ventricles. The latency before the manifestation of the effects would speak for its partial deamination in the periphery with consecutive re-entrance into the brain as 6-azauridine. The penetration of 6-azauridine into the central nervous system is very low,[10, 11] but the cat has been found very susceptible to the toxic effects of 6-azauridine.[9] For this reason it cannot be excluded also that the much lower inhibitory activity of 6-azacytidine-5′-monophosphate on orotidylic acid decarboxylase is sufficient to affect a very susceptible species, especially when 6-azacytidine is injected closely to the site of action.

As deamination of 6-azacytidine occurs in the majority of species hitherto studied,[9] including man,[12] our findings thus support the view that 6-azacytidine acts mainly through its deamination product 6-azauridine. Furthermore, these results, extending our previous findings on the central effects of 6-azauracil and 6-azauridine,[3] bring additional evidence for possible correlations between the metabolism of pyrimidine nucleotides and certain functions of the central nervous system.

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REFERENCES

1. A. D. WELCH, R. E. HANDSCHUMACHER and J. J. JAFFE, *J. Pharmac. exp. Ther.* **129**, 262 (1960).
2. W. WELLS, D. GAINES and H. KOENIG, *J. Neurochem.* **10**, 709 (1964).
3. I. JANKŮ, M. KRŠIAK, L. VOLICER, R. ČAPEK, R. SMETANA and J. NOVOTNÝ, *Biochem. Pharmac.* **14**, 1525 (1965).
4. F. ŠORM, J. SMRT and V. ČERNĚCKIJ, *Experientia* **17**, 64 (1961).
5. R. E. HANDSCHUMACHER, J. ŠKODA and F. ŠORM, *Coll. Czechoslov. Chem. Comm.* **28**, 2983 (1963).
6. J. LÁT, E. M. WIDDOWSON and R. A. MCCANCE, *Proc. R. Soc.* **B153**, 347 (1960).
7. V. KREBS, *Proc. XXII. Internat. Physiol. Congr. Leyden*, Vol. II, DFT 25 (1962).
8. W. FELDBERG and S. L. SHERWOOD, *J. Physiol.* **120**, 3 (1953).
9. J. NOVOTNÝ, R. SMETANA and H. RAŠKOVÁ, *Biochem. Pharmac.* **14**, 1537 (1965).
10. V. HABERMANN and F. ŠORM, *Coll. Czechoslov. Chem. Comm.* **23**, 2201 (1958).
11. S. S. CARDOSO and D. SYMMES, *Fedn Proc. Fedn Am. Socs exp. Biol.* **20**, 322 (1961).
12. Z. MÍSAŘOVÁ and R. SMETANA, unpublished results.