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SHORT COMMUNICATIONS

Potentiation of barbiturate hypnosis by 5-diazoimidazole-4-carboxamide and its derivatives

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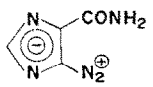
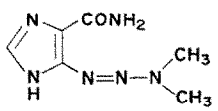
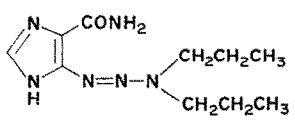
WE HAVE previously reported that 5-diazoimidazole-4-carboxamide (diazo-ICA) has multiple pharmacological and biochemical effects, such as flattening of the electroencephalogram (EEG), depression of the blood pressure, reduction of body temperature and inhibiting diuresis.[1–5]* Recently, we synthesized various thioazoimidazoles from diazo-ICA, and found that they were more stable and potent antitumor agents than diazo-ICA.† These newly synthesized compounds, like diazo-ICA,



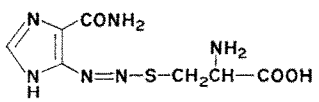
5-Diazoimidazole-4-carboxamide (Diazo-ICA)



5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (Dimethyltriazeno-ICA)‡



5-(3,3-Dipropyl-1-triazeno)imidazole-4-carboxamide (Dipropyltriazeno-ICA)‡



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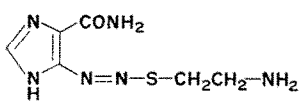
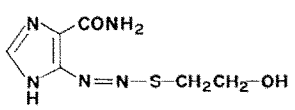
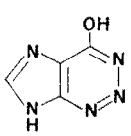


5-(2-Aminoethylthioazo)imidazole-4-carboxamide (Aminoethylthioazo-ICA)‡



5-(2-Hydroxyethylthioazo)imidazole-4-carboxamide (Hydroxyethylthioazo-ICA)



2-Azahypoxanthine

FIG. 1. Chemical structures of diazo-imidazole derivatives.

* H. Iwata and I. Yamamoto, unpublished data.
† H. Iwata, E. Gohda and I. Yamamoto, unpublished data.
‡ These compounds were used as hydrochloride forms.

were shown to be potent inhibitors of xanthine oxidase and uricase.[6,7] On injection into animals these derivatives had sedative effects, such as inhibition of motor activity, although they had no hypnotic action themselves, even at a high dose. A wide variety of centrally acting depressants, including chlorpromazine, reserpine and many other compounds differing in chemical structures and pharmacological effects prolong the period of sleep induced by barbiturates. Accordingly we examined the effects of diazo-ICA and its thioazo compounds on hypnosis induced by hexobarbital. This communication describes the effects of diazo-ICA, two related triazene compounds and three thioazo derivatives on hexobarbital-induced hypnosis and the possible mechanisms involved are discussed.

The chemical structures of these compounds are shown in Fig. 1. These compounds were injected intraperitoneally into male dd-strain mice weighing about 20 ± 1 g. The duration of hypnosis was determined by measuring the period of loss of the righting reflex. The activity of the enzyme metabolizing hexobarbital in the 9000 *g* supernatant fraction of liver was measured as described by Fouts and Brodie.[8] Hexobarbital was determined by the method of Cooper and Brodie.[9]

TABLE 1. POTENTIATION OF HEXOBARBITAL-INDUCED SLEEP BY DIAZO-IMIDAZOLE DERIVATIVES

Compound (30 min pretreatment)	Sleeping time (min ± S. E.)	Control (%)
None	29 ± 1·9 (22)	100
Diazo-ICA; 5 mg/kg, i.p.	73 ± 4·9 (5)*	252
Dimethyltriazeno-ICA; 100 mg/kg, i.p.	34 ± 2·9 (6)	117
Dipropyltriazeno-ICA; 100 mg/kg, i.p.	63 ± 5·9 (5)*	217
Aminocarboxyethylthioazo-ICA 100 mg/kg, i.p.	95 ± 11·8 (6)*	328
Aminoethylthioazo-ICA; 100 mg/kg, i.p.	158 ± 8·8 (5)*	545
Hydroxyethylthioazo-ICA; 100 mg/kg, i.p.	212 ± 8.8 (6)*	731
2-Azahypoxanthine; 100 mg/kg, i.p.	36 ± 2·3 (5)	124

Hexobarbital-Na: 60 mg/kg, i.p.
* Significant difference from control: $P < 0.01$.

Table 1 shows the effects of diazo-imidazole derivatives on the duration of sleep induced by hexobarbital. In mice pretreated with diazo-ICA (5 mg/kg), the hypnosis elicited by sodium hexobarbital (60 mg/kg) lasted for 73 min, or about 250 per cent of the period in control animals. Dipropyltriazeno-ICA (100 mg/kg) prolonged the period to about 200 per cent of that in control animals while aminocarboxyethylthioazo-ICA, aminoethylthioazo-ICA and hydroxyethylthioazo-ICA had more marked actions causing 300, 500 and 700 per cent increase in the period, respectively. Oral administration of diazo-ICA, dipropyltriazeno-ICA, aminocarboxyethylthioazo-ICA, aminoethylthioazo-ICA or hydroxyethylthioazo-ICA also potentiated hypnosis by hexobarbital. 2-Azahypoxanthine, a derivative of diazo-ICA with a fused ring, and dimethyltriazeno-ICA had no effect on the barbiturate hypnosis. Moreover, the structural analog of diazo-ICA, 3-diazo-4-pyrazolecarboxamide and its derivative with a fused-ring, 4-hydroxypyrazolo (3,4-d)-v-triazine, which were both synthesized in our laboratory by the method of Cheng *et al.*,[10] also had no effect.

TABLE 2. EFFECTS OF DIAZO-IMIDAZOLE DERIVATIVES ON THE LIVER ENZYME METABOLIZING HEXOBARBITAL

Treatment	Enzyme activity* (μmoles/g/hr)	Control (%)
None	2·18 ± 0·26 (5)	100
Dipropyltriazeno-ICA		
100 mg/kg, i.p.	1·21 ± 0·18 (5)	55
Aminoethylthioazo-ICA		
50 mg/kg, i.p.	2·23 ± 0·34 (5)	102
100 mg/kg, i.p.	2·14 ± 0·28 (5)	98

* Mean ± S. E.
Animals were killed 1 hr after treatment.
Enzyme preparation: liver 9000 *g* supernatant fraction.

Dipropyltriazeno-ICA (100 mg/kg) reduced the activity of the liver enzyme metabolizing hexobarbital by 55 per cent in 1 hr, but the thioazo compound, aminoethylthioazo-ICA had no effect on this enzyme, when given at a dose of 50 or 100 mg/kg (Table 2).

The effects of diazoimidazole derivatives on the rate of biotransformation of hexobarbital are shown in Fig. 2. Pretreatment with dipropyltriazeno-ICA delayed the disappearance of hexobarbital from the brain, but aminoethylthioazo-ICA had no effect. On administration of dipropyltriazeno-ICA hypnosis was prolonged but the brain hexobarbital level when animals regained their righting reflex was not significantly different from that of control animals. However, after pretreatment with aminoethylthioazo-ICA, the concentration of the hypnotic in the brain was lower than that in control animals on awakening.

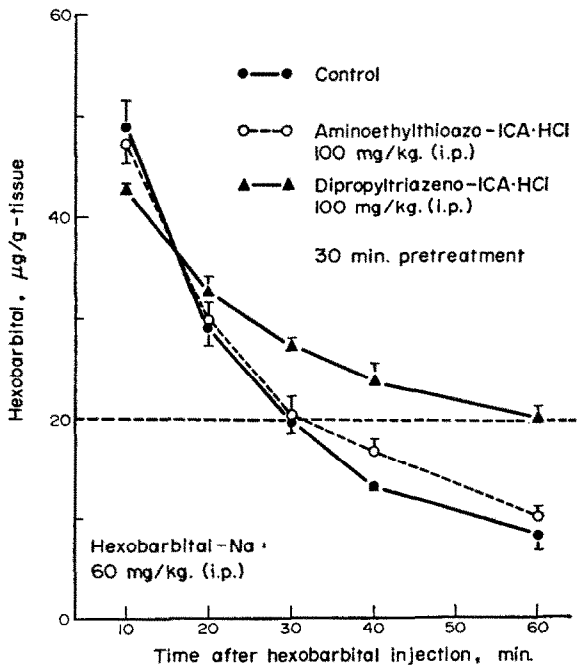


FIG. 2. Effects of diazo-imidazole derivatives on the concentration of hexobarbital in mouse brain.

These results suggest that prolongation of hexobarbital-induced sleep by dipropyltriazeno-ICA may be due to the inhibition of the metabolism of hexobarbital. On the other hand aminoethylthioazo-ICA probably potentiates barbiturate hypnosis by making the animals more sensitive to the hypnotic.

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Department of Pharmacology,
Faculty of Pharmaceutical Sciences,
Osaka University,
Toneyama, Toyonaka,
Osaka, Japan

KYOJI MORITA
ITARU YAMAMOTO
HEITAROH IWATA

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Effect of Persantin on nucleoside metabolism of the perfused rabbit heart*

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PERSANTIN [2,6-bis(diethanolamine)-4,8-dipiperidinopyrimindo(5,4-*d*)pyrimidine] has been used clinically as a vasodilator.[1,2] The basis of this effect is not known; however, it has been suggested that the drug acts to spare adenosine, a potent vasodilator, from dismutation. Persantin inhibition of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) is well recognized.[3] On the other hand, the vasodilator effect of administered adenosine is potentiated at dose levels of Persantin far below those required to inhibit adenosine deaminase activity (10^{-4} M).[3] Adenosine is avidly incorporated into the heart, and it has been suggested that its rapid uptake may be the basis for the termination of its vasodilator action.[4] Persantin inhibits the uptake of adenosine into red blood cells[5] and into the myocardium,[6] and it is this effect which may explain the potentiation of adenosine-induced vasodilation. Most of the studies on Persantin inhibition of adenosine uptake have dealt with the total radioactivity in the tissue. Thus, the effect of Persantin on the fate of adenosine taken up by the heart has not been previously reported. The present study is concerned with the effect of Persantin on the uptake of [8-^{14}C]adenosine and [8-^{14}C]inosine into the perfused rabbit heart and on the incorporation of the labeled nucleosides into the myocardial adenine nucleotides.

The methods used in this experiment have been described in a previous paper.[4] Rabbit hearts were perfused at 25 ml/min on a non-recirculating basis for 30–60 min with Ringer's solution equilibrated with 95% O_2 and 5% CO_2 at 31°. The perfusion medium was then changed to the Ringer's solution containing labeled adenosine or inosine, and the hearts were perfused for an additional period of 3 or 30 min. Persantin was made up to the desired concentrations in nucleoside containing Ringer's and perfused through the heart together with the labeled nucleoside. At the end of perfusion, the heart was freeze-pressed between the jaws of an aluminium clamp precooled in liquid N_2 and the radioactivity in the heart powder and in the perchloric acid extract was determined. Acid soluble nucleotides and their metabolites were separated by anion exchange column chromatography.[7] The concentration of nucleotides was measured with a spectrophotometer according to the procedure of Kalckar[8] and the radioactivity was determined with a liquid scintillation counter. Nucleotide specific activity is expressed as counts/minute per micromole of the nucleotide.

Persantin added to the perfusion medium at the concentration of 0·25–1·0 μg/ml greatly decreases the specific activity of adenine nucleotides determined after 30 min of perfusion with 0·2 μM of [8-^{14}C]adenosine (Fig. 1). The levels of myocardial adenine nucleotides are not affected. The lowest concentration (0·25 μg/ml) suffices to reduce the specific activity by 80 per cent. Concentrations of the drug greater than 0·25 μg/ml have little effect on the extent of inhibition of adenosine uptake. On the other hand, the order of magnitude of specific activity among adenine nucleotides (i.e. ADP ≥ ATP ≥ AMP) is not altered by Persantin. The total radioactivity recovered from the Persantin-treated hearts is consistently lower than that seen in the absence of the drug, while the distribution of the counts in the heart remains unchanged (Table 1). In both cases, the majority of the counts is found in the compartments extractable by perchloric acid, and the activity in the acid extract is mostly associated with the adenine nucleotides. The inhibition of adenosine uptake occurs so rapidly that lower specific activity of adenine nucleotides is observed even after 3 min of perfusion.

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