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Dose-dependence of the effect of hydralazine on the central nervous system in rats

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The pharmacological action of hydralazine has been reviewed by several authors (Koch-Weser 1976; Israili & Dayton 1977; Koch-Weser 1978). However, little work has been done on the central actions of hydralazine, since it is generally assumed that hydralazine exerts a direct action on blood vessels (Khayyal et al 1981) by inhibition of calcium release in the vessels (Lipe & Moulds 1981) or mediation of vasodilating prostaglandins (Cangiano et al 1978; Haeusler & Gerold 1979), resulting in a reduction in blood pressure. Our previous paper (Satoh et al 1981) indicated that hydralazine elicited convulsions at high doses (>40 mg kg^{-1}) in rats, and that changes in the central γ-aminobutyric acid (GABA) system might be involved in the adverse effect of hydralazine. Later, we demonstrated that a lower dose (10 mg kg^{-1}) of hydralazine potentiated the hypnotic effects of hexobarbitone (without any inhibition of hepatic drug metabolism) and of intracerebroventricularly administered phenobarbitone in rats (Hara et al 1981). These findings suggest that hydralazine can act on the central nervous system, but it is not clear whether or not the action at lower doses of hydralazine occurs at the same site as that at higher doses. The present work was carried out to investigate the central effects of hydralazine at high and low doses by studying its effect on seizure-induced by two different central nervous stimulants, leptazol (pentylenetetrazol) and picrotoxin, in rats.

Method

Male Wistar rats, 180–200 g, were subcutaneously injected with leptazol (100 mg kg^{-1}) or picrotoxin (8 mg kg^{-1}) 60 min after a dose of hydralazine (10 or 40 mg kg^{-1} i.p.), since pilot studies indicated that these doses of the central stimulants could evoke almost the same seizure severity. Hydralazine at a dose of 40 mg kg^{-1}, which was considered to be the threshold dose able to cause seizure, as previously reported (Satoh et al 1981), evoked seizure in 10 to 15% of animals without the stimulants. Therefore, we excluded them from the data in this study. Control animals received equal volumes of the vehicle (0·9% NaCl, saline). Behavioural changes following administration of leptazol or picrotoxin were continuously observed for 60 min, according to the following index of seizure severity as reported by Satoh et al (1979). No behavioural change, 0; myoclonus, 1; myoclonus and mild clonic seizure, 2; myoclonus, mild and severe clonic seizure, 3; myoclonus, mild clonic, severe clonic and tonic seizure, 4; death, 5.

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Results

Fig. 1 shows the effects of hydralazine at low and high doses on the seizure severity score after leptazol injection. The lower dose of hydralazine significantly reduced the score until 45 min after leptazol (Fig. 1A). Although at the higher dose, the score was not significantly changed, slight enhancement in the score was observed after leptazol as compared with the control (Fig. 1B). Fig. 2 shows the effects of hydralazine on picrotoxin-induced seizure. In contrast to the results using leptazol (Fig. 1), neither enhancement nor reduction of the seizure severity score after picrotoxin on pretreatment with the lower dose of hydralazine was observed (Fig. 2A). However, significant potentiation

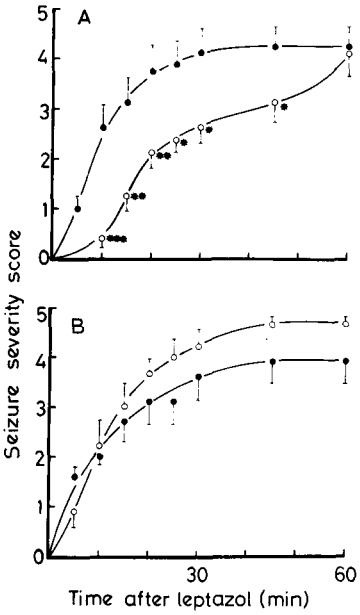

FIG. 1. Effects of hydralazine at low (10 mg kg^{-1}, A) and high (40 mg kg^{-1}, B) doses on the seizure severity score following injection of leptazol. Hydralazine was administered intraperitoneally 60 min before the injection of leptazol (100 mg kg^{-1} s.c.). Closed and open circles represent control and hydralazine-treated groups, respectively. Vertical bars indicates standard errors of the means of 8 to 10 animals. Statistical significance in Student's *t*-test: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

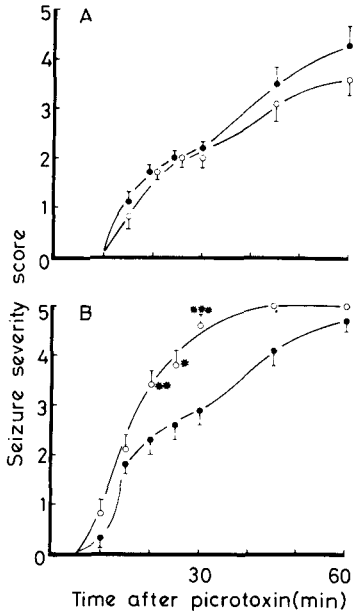

FIG. 2. Effects of hydralazine at low (10 mg kg^{-1}, A) and high (40 mg kg^{-1}, B) doses on the seizure severity score following injection of picrotoxin. Picrotoxin (8 mg kg^{-1} s.c.) was injected 60 min after hydralazine. For details, see the legend to Fig. 1.

of picrotoxin-induced seizure was seen in the animals treated with hydralazine at the higher dose (Fig. 2B).

Discussion

Data in this paper clearly show that the higher dose of hydralazine significantly enhanced picrotoxin-induced seizure, and that the lower dose of hydralazine reduced the seizure evoked by leptazol. It is well established that the central GABA-ergic system, which plays a role in inhibitory neurotransmission (Enna & Maggi 1979), is involved in the seizure following picrotoxin treatment (Löscher & Frey 1977), because picrotoxin inhibits GABA synthesis (Löscher & Frey 1977), and further, it affects GABA-activated chloride ionophore, resulting in a reduction of GABA synaptic activity (Andrews & Johnston 1979). Our previous findings (Satoh et al 1981) suggested that the seizure caused by hydralazine at high doses might be related to changes in the central GABA system, and are, therefore, confirmed by the present results. Hydralazine at the higher dose used here slightly, but not significantly, enhanced leptazol-induced seizure. However, this enhancement may be in part due to interference by hydralazine with the hepatic drug metabolizing enzyme system which makes leptazol inert (Wohland & Koransky 1972), since we recently found that hydralazine inhibited this enzyme system at this dose in rats (Hara et al 1982). On the other hand, the lower dose of hydralazine significantly reduced the seizure following injection of leptazol, but not picrotoxin. Accumulating evidence indicates that leptazol-induced seizure may be related to central monoaminergic systems (Chen et al 1954; Little & Conrad 1960; Kilian & Frey 1973). Therefore, the action of hydralazine at the lower dose might be correlated with changes in central monoaminergic systems, but not the GABA-ergic system, because there were no changes in picrotoxin-induced seizure in the animals pretreated with hydralazine at the lower dose, as compared with those in the control.

In conclusion, it appears that hydralazine can affect the central nervous system in different ways at low and high doses. From the present results, it is reasonable to conclude that the effect of a high dose of hydralazine in rats may be related to the central GABA-ergic system rather than to monoaminergic systems. On the other hand, it is now difficult to interpret the transient antagonistic action of a low dose of hydralazine against leptazol-induced seizure, because it is considered that complex factors mediating central neurotransmissions are involved in the seizure (de la Torre & Mullan 1970; Corcoran et al 1973; Kilian & Frey 1973; Diaz 1974; McMillen & Isaac 1978; Oishi et al 1979). However, we can exclude the mediation of the central GABA-ergic system in this antagonistic action of hydralazine against the seizure evoked by leptazol.

REFERENCES

Andrews, P. R., Johnston, G. A. R. (1979) Biochem. Pharmacol. 28: 2697–2702

Cangiano, J. L., Rodriguez-Sargent, C., Martinez-Maldonado, M. (1978) J. Lab. Clin. Med. 92: 516–520

Chen, G., Ensor, C. R., Bohner, B. (1954) Proc. Soc. Exp. Biol. N.Y. 86: 507–510

Corcoran, M. E., Fibiger, H. C., McGeer, E. G., Wada, J. A. (1973) J. Pharm. Pharmacol. 25: 497–499

de la Torre, J. C., Mullan, S. (1970) Ibid. 22: 858–859

Diaz, P. M. (1974) Neuropharmacology 13: 615–621

Enna, S. J., Maggi, A. (1979) Life Sci. 24: 1724–1738

Haeusler, G., Gerold, M. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 310: 155–167

Hara, S., Satoh, T., Kitagawa, H. (1981) Res. Commun. Chem. Pathol. Pharmacol. 31: 251–257

Hara, S., Satoh, T., Kitagawa, H. (1982) Ibid. 36: 260–272

Israili, Z. H., Dayton, P. G. (1977) Drug. Met. Rev. 6: 283–305

Khayyal, M., Gross, F. V., Kreye, A. W. (1981) J. Pharmacol. Exp. Ther. 216: 390–394

Kilian, M., Frey, H.-H. (1973) Neuropharmacology 12: 681–692

Koch-Weser, J. (1976) New Engl. J. Med. 295: 320–323

Koch-Weser, J. (1978) Am. Heart J. 95: 1–3

Lipe, S., Moulds, R. F. W. (1981) J. Pharmacol. Exp. Ther. 217: 204–208

Little, J. M., Conrad, E. A. (1960) Ibid. 129: 454–461

Löscher, W., Frey, H.-H. (1977) Naunyn-Schmiedeberg's Arch. Pharmacol. 296: 263–269

McMillen, B. A., Isaac, L. (1978) Biochem. Pharmacol. 27: 1815–1820

Oishi, R., Suenaga, N., Hidaka, T., Fukuda, T. (1979) J. Pharm. Pharmacol. 31: 709–710

Satoh, T., Fukumori, R., Nakagawa, I., Minegishi, A., Kitagawa, H., Yanaura, S. (1979) Res. Commun. Chem. Psychol. Psychia. Behav. 4: 285–297

Satoh, T., Hara, S., Numata, Y., Kitagawa, H. (1981) Biochem. Pharmacol. 30: 1155–1157

Wohland, H. W., Koransky, W. (1972) Naunyn-Schmiedeberg's Arch. Pharmacol. 273: 99–108

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The effect of adrenalectomy on hepatic mixed function oxidase activity in female rats

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It has been demonstrated that the livers of adrenalectomized male rats have an impaired ability to metabolize Type I drug substrates of the cytochrome P450-dependent mixed function oxidase (MFO) system whereas the ability to metabolize Type II substrates is unaffected (Kato & Gillette 1965; Marshall 1971; Stripp et al 1971). On the other hand, it has been reported that the livers of adrenalectomized female rats show no such defect in the ability to metabolize Type I substrates (Kato & Gillette 1965; Stripp et al 1971), and it has been suggested that this effect of adrenalectomy in male rats is mediated through impairment of the androgen-stimulating effects known to occur in male rats (Kato 1977). As the sex difference in drug metabolism is believed to be confined to the rat and, to a much lesser extent, the mouse, it has been suggested from the results with the female rats that humans with adrenal insufficiency are unlikely to have an impaired hepatic ability to metabolize drugs (Kato 1977). The male and female rats used in these early investigations were studied 4 days post adrenalectomy but as it is known that there are sex differences in the turnover of hepatic cytochrome P450 in the rat (female rats not showing the rapid first phase of cytochrome P450 degeneration observed in male rats, Levin et al 1975), it was considered possible that this period was too short to demonstrate conclusively the effect of adrenalectomy on the MFO system of female rats. We have therefore studied in-vivo and in-vitro certain aspects of the hepatic MFO system in female rats 7–8 days post adrenalectomy, using typical Type I (7-ethoxycoumarin (7EC), hexobarbitone (HB)) and Type II (*p*-chloro-*N*-methylaniline (PCMA)) substrates. We have also studied the longer-term effects of adrenalectomy, and for this reason some animals were maintained for 47–50 days post adrenalectomy before measurement of hepatic MFO activity.

Method

Female Wistar rats (initially ca 100 g) were obtained from the University of Nottingham Joint Animal Breeding Unit, Sutton Bonington, Leics, U.K. Bilateral adrenalectomy was performed under halothane anaesthesia and control rats were sham-operated under identical conditions. The animals were left for 7–8 or 47–50 days and throughout this time they were allowed free access to standard laboratory diet and 1% NaCl in the drinking water (to compensate for loss of sodium ions due to loss of adrenals and, hence, mineralocorticoid control). Liver 10 000 *g* supernatants and microsomal fractions were prepared as described previously (Litterst et al 1975; Fry 1981) and assayed for protein and cytochrome P450 content, and 7-EC *O*-de-ethylase and PCMA *N*-demethylase activities by published methods (Lowry et al 1951; Joly et al 1975; Greenlee & Poland 1978; Aitio 1978; Kupfer & Bruggeman 1966).

In the sleeping time studies the animals were injected i.p. with HB at 100 mg kg^{-1} in water adjusted to pH 10·2 with NaOH, and the sleeping time was recorded from the loss to the recovery of the righting reflex. Throughout the sleeping time the animals were maintained at 37 °C by being kept on a heated table.

Success of the adrenalectomy was checked by visual examination immediately after death. Statistical comparison of the results was performed by means of an unpaired Student's *t*-test (Campbell 1967).

Table 1. Body and liver weights and hepatic MFO activity of female Wistar rats 7–8 days after sham-operation ('Control rats') or adrenalectomy.

	Control rats	Adrenalectomized rats
Body weight (g)	126·5 ± 1·7 (17)	126·6 ± 1·2 (19)
Liver weight (g)	5·97 ± 0·17(12)	4·93 ± 0·14(12)***
Cytochrome P450 content (nmol g^{-1} liver)	4·50 ± 0·42(6)	3·00 ± 0·27(6)**
7-EC *O*-de-ethylase activity (nmol product g^{-1} liver min^{-1})	15·07 ± 2·43(6)	8·67 ± 1·47(6)*
(nmol product $nmol^{-1}$ P450 min^{-1})	3·48 ± 0·56	2·89 ± 0·46
PCMA *N*-demethylase activity (nmol product g^{-1} liver min^{-1})	92·6 ± 6·5(6)	61·2 ± 6·9(6)***
(nmol product $nmol^{-1}$ P450 min^{-1})	21·0 ± 1·3	20·3 ± 1·5
HB sleeping time (min)	43·2 ± 3·1(5)	72·4 ± 5·3(7)***

Values are mean ± s.e. with the numbers of rats given in brackets. Test values significantly different from control values at $*P < 0.05$; $**P < 0.02$. $***P < 0.01$.

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