altogether these data indicate a regional difference on GABA action and that bicuculline sensitive receptors ($GABA_A$ receptors) are present at ganglionic level probably both in motor- and inter-neurons.

REFERENCES

Costa, M., Furness, J. B. (1979) Br. J. Pharmacol. 65: 237–248

Furness, J. B., Costa, M. (1973) Philos. Trans. R. Soc. London Ser. B 265: 123–133

Gershon, M. D. (1981) Ann. Rev. Neurosci. 4: 227–272

Huidobro-Toro, J. P., Foree, B. (1980) Eur. J. Pharmacol. 61: 335–345

Krantis, A., Kerr, D. I. B. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 317: 257–261

Krantis, A., Costa, M., Furness, J. B., Orbach, J. (1980) Eur. J. Pharmacol. 67: 461–468

Tonini, M., Onori, L., Lecchini, S., Frigo, G. M., Perucca, E., Saltarelli, P., Crema, A. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 324: 180–184

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Monoamine oxidase inhibition by the tremorogenic drug—LON 954

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N-Carbamoyl-2-(2,6-dichlorophenyl)acetamidine HCl (LON 954), a tremorogenic drug, inhibited MAO activity in various tissue preparations in a reversible, competitive manner showing some degree of selectivity towards type-B MAO.

The tremorogenic drug LON 954 (*N*-carbamoyl-2-(2,6-dichlorophenyl) acetamidine HCl) (Coward et al 1977) potentiates the effects of some biogenic monoamines, e.g. tyramine, in in-vitro pharmacological studies. This prompted us to investigate whether it has any monoamine oxidase (MAO) inhibitory activity.

Methods

Mitochondrial preparations from various tissues were used as the enzyme source. Rat brain mitochondrial preparations, with either MAO type selectively inhibited, were obtained as described by Mitra & Guha (1980). Rat liver mitochondrial preparations with one MAO type selectively inhibited in-vitro, were prepared according to Tipton et al (1982) with slight modification. Rat liver mitochondrial suspensions were preincubated with either 0·3 μM clorgyline (37 °C for 60 min) or 0·3 μM selegiline (deprenyl) (37 °C for 90 min) to effect complete titration MAO-A or -B respectively. Then after centrifugation at 20000*g* and resuspension the mitochondrial pellet now showed exclusive activity of one MAO type, tyramine deamination was assayed by estimating aldehyde production as described by Mitra & Guha (1978). In some preliminary studies MAO activity was also assayed by manometric measurement of oxygen uptake (Creasy 1956) or spectrophotometric measurement of benzaldehyde production (Turski et al 1973).

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Results and discussion

LON 954 inhibited MAO activity in different tissue preparations with tyramine as substrate (Table 1). The degree of inhibition was observed to vary from preparation to preparation which may have some relationship with the type of MAO involved in the reaction. With preparations showing exclusively or predominantly MAO-A activity, a moderate degree of inhibition was observed. With the type-B rich preparations the inhibition was more marked. That this observed inhibition is enzyme inhibition and not an artifact of the assay procedure was confirmed by manometric measurement of oxygen uptake and spectrophotometric measurement of benzaldehyde production, respectively.

The possibility of some discrimination between the two MAO types by LON 954 (Table 1), was explored

Table 1. In-vitro inhibition by LON 954 of MAO activity in different tissue preparations. Crude mitochondrial preparations of each tissue was used as enzyme source. There was a 10 min preincubation of the tissue preparation with 10^{-3} M (final concentration) LON 954 in an otherwise complete reaction mixture before addition of substrate (0·01 M tyramine). For each tissue preparation, enzyme concentration and incubation period were chosen within the linear range.

Tissue preparation	MAO	Per cent inhibition of MAO
Rat liver	MAO A and B	70
Brain tissue from rats pretreated with clorgyline	B	94
Brain tissue from rats pretreated with pargyline	A	45
Rabbit liver	B predominant	99
Guinea-pig brain	B predominant	82
Guinea-pig kidney	A predominant	57

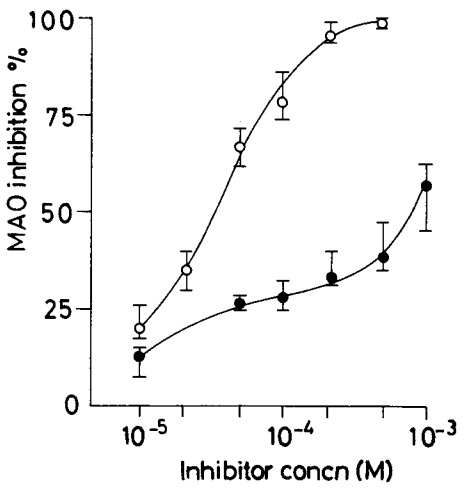

FIG. 1. In-vitro inhibition of tyramine deamination by LON 954. Mitochondrial preparations of guinea-pig kidney (—●—) or rabbit liver (—○—) were preincubated with various concentrations of the inhibitor for 10 min at 37 °C before addition of substrate. Each point is the mean of three determinations; the bar gives the range.

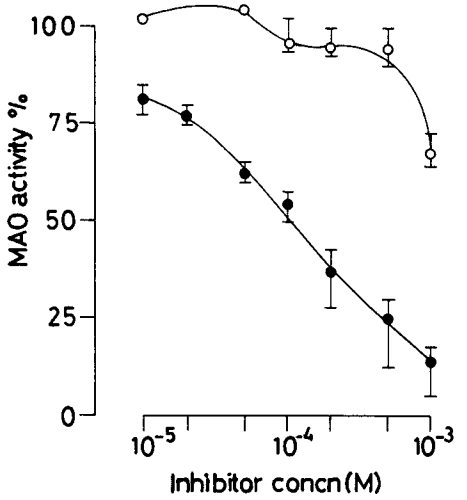

FIG. 2. In-vitro inhibition of tyramine oxidation by rat liver MAO types in the presence of various concentrations of LON 954. Samples of selectively inhibited rat liver mitochondrial preparation showing exclusively type A (—○—) or type B (—●—) activity were preincubated for 10 min at 37 °C with the inhibitor in an otherwise complete reaction mixture before addition substrate. Each point is the mean of three determinations; the bar gives the range.

using guinea-pig kidney and rabbit liver mitochondrial preparations which are rich in type-A and type-B MAO respectively (Fig. 1). The two preparations showed a pronounced difference in their sensitivities towards LON 954. With guinea-pig kidney mitochondrial MAO, a fifty-fold increase in inhibitor concentration (from 10^{-5} to 5×10^{-4} M) did not produce any remarkable increase in MAO inhibition, this region of the curve resembling a plateau followed by a steep rise. The dose-response curve for rabbit liver MAO exhibited a steep rise in the same inhibitor concentration region, inhibition being virtually complete at the higher inhibitor doses used. This difference in the sensitivity of MAO-A and -B to LON 954, is further confirmed by the dose-response curves of two selectively inhibited rat liver preparations (Fig. 2). Little inhibition of MAO-A occurred at LON 954 0·5 mM, which did, however, effect >75% inhibition of MAO-B.

With a mouse liver mitochondrial preparation, which is predominantly MAO-B [Squires' (1972) observation, with kynuramine as substrate, was confirmed with tyramine as substrate in a clorgyline inhibition plot (unpublished observation in this laboratory)], reversibility of the inhibition was tested by the method of Ackermann & Potter (1949). Product formation plotted against enzyme concentration in the presence and absence of the inhibitor produced two straight lines which converged at zero indicating reversible inhibition. This was confirmed by a dilution experiment and also by the lack of any preincubation effect on the degree of inhibition by a submaximal dose of the inhibitor.

Fig. 3 shows the Lineweaver & Burk (1934) plots of mouse liver mitochondrial MAO at different concentra-

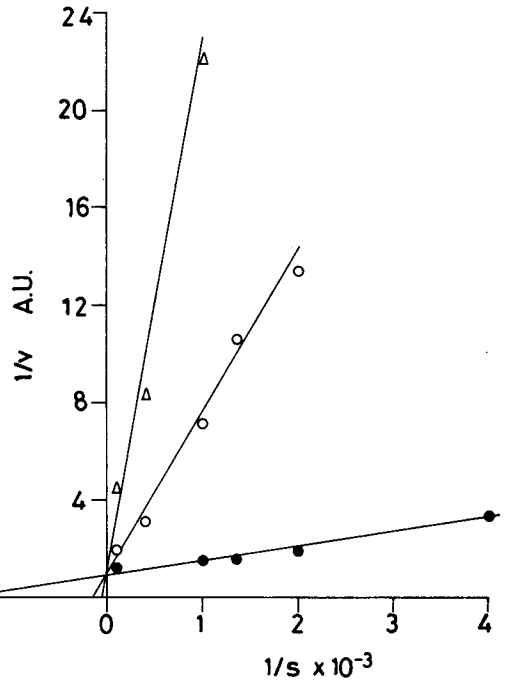

FIG. 3. Lineweaver-Burk Plot showing inhibition of mouse liver MAO by LON 954 at 0 (—●—), 100 (—○—) and 500 (—△—) μM respectively. Various concentrations of tyramine were used as substrate and no preincubation of the enzyme with the inhibitor was allowed. Each point is the mean of duplicate determinations.

Table 2. K_i values of LON 954 for MAO inhibition with various tissue preparations. K_i values were determined from Dixon (1953) plots, each is the average of three determinations.

Tissue preparation	MAO	K_i value (μM)
Brain mitochondria from pargyline-treated rats	A	45
Brain mitochondria from clorgyline-treated rats	B	8
Rat liver mitochondria pre-treated in-vitro with 0·3 μM selegiline	A	38
Rat liver mitochondria pre-treated in-vitro with 0·3 μM clorgyline	B	7
Mouse liver mitochondria	B predominant	10
Rabbit liver mitochondria	B predominant	4

tions of tyramine obtained with LON 954 concentrations of 0, 100 and 500 μM. The three lines intercept on the ordinate indicating competitive inhibition. Similar plots were obtained with brain mitochondrial preparation from rats pretreated with clorgyline to selectively inhibit MAO-A.

The competitive nature of MAO inhibition by LON 954 was confirmed by Dixon (1953) plots (not shown) using different tissue preparations (Table 2). As expected from the dose-response curves, tissue preparations rich in MAO-B show lower K_i values for this inhibitor, although the difference in K_i values is not as much as might have been expected from the dose-response patterns. As LON 954 is a competitive inhibitor, the IC50 value depends on the substrate concentration used. The tyramine concentration in our study probably represents different multiples of the K_m value for the different tissue preparations which may explain the greater selectivity exhibited by LON 954 in dose-response patterns.

Although the tremorogenic property of LON 954 and related compounds was discovered comparatively recently (Coward et al 1977), many compounds carrying the amidino group have long been known to have MAO inhibitory properties (Bernheim 1943; Blaschko & Duthie 1945; Fastier & Hawkins 1951; Blaschko & Himms 1955). One of these compounds, pentamidine, inhibited rabbit liver MAO 50 times more than guinea-pig liver MAO (Blaschko & Himms 1955). On the basis of our present knowledge of MAO, this can be interpreted as being due to a preference on the part of the inhibitor towards MAO-B, which accounts for most of the enzyme activity in rabbit liver (Squires 1972). Blaschko & Himms (1955) saw this as species variation. MAO inhibitory potency differed among the compounds tested and the degree of reversibility of inhibition depended upon the chain length in a particular homologous series. With the lower members, inhibition was usually weak and easily reversible which supports our observations with LON 954 which has a short chain length separating the amidino group from the aromatic ring.

The tremor produced by LON 954 appears to involve some central dopaminergic pathways (Coward et al 1977). In view of the reversible nature of the MAO inhibition by LON 954, the rapid onset of tremors suggests that the two pharmacological effects may be unrelated. Another reversible MAO inhibitor, harmine is also known to have tremorogenic activity (Neuner & Tappeiner 1895) which is possibly effected by some interference with central dopamine mechanisms. However, the harmala alkaloids are selective for MAO-A rather than MAO-B. In most animals dopamine is known to be a substrate for both forms of MAO (Neff & Yang 1974) which may preclude any selective MAO inhibitor being able to effect a substantial rise in dopamine concentrations in-vivo, yet at tremorogenic concentrations the drug may be expected to inhibit MAO-B considerably, particularly in the presence of the low amine concentrations encountered in-situ.

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REFERENCES

Ackermann, W. W., Potter, V. R. (1949) Proc. Soc. Exp. Biol. Med. 72: 1–9

Bernheim, F. (1943) Science 98: 223

Blaschko, H., Duthie, R. (1945) Biochem. J. 39: 347–350

Blaschko, H., Himms, J. M. (1955) Br. J. Pharmacol. 10: 451–453

Coward, D. M., Doggett, N. S., Sayers, A. C. (1977) Arzneim.-Forsch/Drug Res. 27: 2326–2332

Creasy, N. H. (1956) Biochem. J. 64: 178–183

Dixon, M. (1953) Ibid. 55: 170–171

Fastier, F. N., Hawkins, J. (1951) Br. J. Pharmacol. 6: 256–262

Lineweaver, H., Burk, D. (1934) J. Am. Chem. Soc. 56: 658–666

Mitra, C., Guha, S. R. (1978) Biochem. Pharmacol. 27: 2455–2457

Mitra, C., Guha, S. R. (1980) Ibid 29: 1213–1216

Neff, N. H., Yang, H. Y. T. (1974) Life Sci. 14: 2061–2074

Neuner, A., Tappeiner, H. (1895) Arch. Exp. Pathol. Pharmakol. 35: 69–80

Squires, R. F. (1972) Adv. Biochem. Psychopharmacol. 5: 355–370

Tipton, K. F., Fowler, C. J., Houslay, M. D. (1982) in: Kamijo, K., Usdin, E., Nagatsu, T. (eds) Monoamine Oxidase Basic and clinical frontiers. Excerpta Medica, Amsterdam, pp 87–99

Turski, W., Turska, E., Gross-Bellard, M. (1973) Enzyme 14: 211–220