Kochevar, I. E., Lamola, A. A. (1979) Photochem. Photobiol. 29: 791–795

Kopecky, J., Shields, J. E. (1971) Collect. Czech. Chem. Commun. 36: 3517–3526

Magnus, I. A. (1976) Dermatological Photobiology, Blackwell, London, p. 213

Rovei, V., Belvedere, G., Pantarotto, C., Frigerio, A. (1976) J. Pharm. Sci. 65: 810–815

Sisenwine, S. F., Tio, C. O., Shrader, S. R., Ruelos, H. W. (1970) J. Pharmacol. Exp. Ther. 175: 51–59

Vogel, A. I. (1956) Practical Organic Chemistry 3rd Ed. p. 894. Longman, London

J. Pharm. Pharmacol. 1984, 36: 48–50
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Biochemical effects of minaprine on striatal dopaminergic neurons in rats

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The biochemical effects of minaprine, a new psychotropic drug, were investigated on striatal dopaminergic neurons in the rat. Minaprine did not displace [^{3}H]spiperone in-vitro binding from striatal membranes but had clear effects on dopamine (DA) metabolites. Homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were significantly decreased in a dose-dependent manner after intraperitoneal administration of minaprine 30 min before killing. In rats injected with minaprine 15 mg kg^{-1} i.p. at different intervals, the decrease in striatal HVA and DOPAC was time-dependent and a concomitant rise in 3-methoxytryramine (3-MT) concentrations was observed. The maximum of these effects was reached 30 min after minaprine. When administered 5 min after a monoamineoxidase (MAO) inhibitor (pargyline, 100 mg kg^{-1} i.p.) and 30 min before killing, minaprine did not affect pargyline-induced changes in HVA, DOPAC and 3-MT levels. This together with other data suggests that minaprine affects DA metabolism by acting, at least partially, at presynaptic level through in-vivo inhibition of MAO activity.

Minaprine (CM30038, morpholinoethylamino-3-methyl-4-phenyl-6-pyridazine) is a novel psychotropic drug synthesized by Wermuth & Exinger (1972); it has convulsant activity and blocks glycine receptors. Biochemical studies have shown that minaprine raises 5-hydroxytryptamine (5-HT) concentration in striatum, hypothalamus, parietal cortex and raphe region. (P. Mandel, personal communication) and induces a marked increase of acetylcholine concentration and a reduction of the dopamine (DA) turnover in the striatum (Garattini 1981).

The mechanism of action on DA metabolism changes induced by minaprine in rat striatum was investigated by measuring its main catabolites: homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) concentrations mainly give an index of the intraneuronal metabolism and 3-methoxytyramine (3-MT), produced in the synaptic cleft, has been proposed as a better indicator of DA release (Carlsson & Lindqvist 1963; Kehr 1976; Di Giulio et al 1978).

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Methods

Male CD-COBS rats (Charles River, Italy) 225–250 g, housed in standard conditions (60% relative humidity, 22 °C, 12-h light-dark cycles) with free access to water and food were injected intraperitoneally with minaprine (kindly supplied by Sanofi, Montpellier, France), at different doses, or pargyline HCl (Aldrich Chem. Comp., USA) 100 mg kg^{-1}, or both. The drugs were dissolved in water and injected in a volume of 2 ml kg^{-1}. Rats were killed at different intervals by microwave irradiation (1·3 W at 2·45 GHz for 4·25 s) to rapidly inactivate catechol-*O*-methyltransferase (COMT). Striata were rapidly dissected, frozen on dry ice and kept at −80 °C until biochemical assay.

For the simultaneous assay of HVA, DOPAC, 3-MT and DA the striata of each animal were homogenized by sonication (Branson Sonifier B 15) in 300 µl of 0·4 M perchloric acid and centrifuged at 10 000 rev min^{-1} for 10 min. The clear supernatant was divided into two parts: 50 µl was used for HVA and DOPAC assay according to a method previously described by Ponzio & Jonsson (1978) and 250 µl was adjusted to pH 7·5–7·6 to separate DA from 3-MT. At this pH, DA was adsorbed onto alumina and 3-MT remained in the supernatant from which it was extracted according to Ponzio et al (1981a). DA was eluted from alumina according to Keller et al (1976). Liquid chromatography with electrochemical detection (LCEC) (Bioanalytical Systems Inc., West Lafayette, Ind.) was used for all biochemical determinations. A glass column 500 mm long, 2 mm i.d., packed with a cation exchange resin (Vydac CX, P310. The Separation Group Hesperia, CA) was used for DA determination. Samples were eluted with citrate-acetate buffer pH 5·2, 0·33 M; detector potential +0·75 V.

HVA and DOPAC were separated in a glass column 750 mm long, 2 mm i.d., packed with anionic exchange resin (Zipax SAX Du Pont) using citrate-acetate buffer pH 7·4, 0·04 M as eluant; detector electrode potential: +0·75 V.

3-MT was separated in a glass column 300 mm long, 2 mm i.d., packed with cation exchange resin Vydac P

Table 1. Effects of different doses of minaprine on striatal DA and its metabolites 30 min after drug administration.

	Minaprine (mg kg^{-1})					
	0	2·5	5	10	15	30
HVA	509 ± 36	387 ± 32	407 ± 22	355 ± 37*	321 ± 34**	312 ± 16**
DOPAC	534 ± 23	403 ± 32**	364 ± 11**	294 ± 12**	239 ± 25**	241 ± 12**
3-MT	15·2 ± 1·2	19·1 ± 1·2	24·5 ± 3·5*	35 ± 2·5**	43·6 ± 2·8**	56·6 ± 2·3**

Data are expressed as ng g^{-1} wet tissue (± s.e., n = 6).
* $P < 0.05$, ** $P < 0.01$, difference from controls by Dunnett's test.

310 (The Separation Group Hesperia, CA) using citrate acetate buffer pH 5.2, 0.12 M as eluant; detector electrode potential: +0·76 V.

In displacement studies [^{3}H]spiperone (New England Nuclear, S.A. 27·6 Ci mmol^{-1}) binding to rat striatal membranes was measured according to Burt et al (1977).

Statistical analysis was with Dunnett's test (Kramer 1956) and two-way analysis of variance.

Results

Minaprine at concentrations up to 5 × 10^{-4} M was unable to displace [^{3}H]spiperone from its binding sites in rat striatal membranes (data not shown), suggesting it does not interact with dopaminergic receptors in this brain area. However, minaprine induced a dose-dependent reduction in striatal concentrations of the acid metabolites HVA and DOPAC (Table 1). DOPAC concentrations were already lowered by the lowest dose tested (2·5 mg kg^{-1}) and the reduction was maximal at 15 mg kg^{-1}. HVA concentrations were less sensitive to minaprine, a statistically significant reduction only being induced by doses of 10 mg kg^{-1} upwards.

In contrast with the reduction of HVA and DOPAC, concentrations of the extraneuronal DA metabolite, 3-MT, were significantly raised as early as 30 min after 5 mg kg^{-1} of minaprine, reaching an almost four-fold increase at the dose of 30 mg kg^{-1}. DA was not affected by minaprine at the doses used (data not shown).

In another experiment the striatal values of DA, HVA, DOPAC and 3-MT were measured 15, 30, 60, 90 and 120 min after injection of minaprine 15 mg kg^{-1} i.p. (Table 2). DA values were not significantly different from controls at any tested time, although they tended to be slightly higher. DOPAC and HVA showed a decrease that peaked at 30 min and was still present 120 min after drug administration. In contrast with the acid metabolites, striatal 3-MT concentrations rose significantly in the interval from 15 to 90 min after injection with the maximum effect at 30 min.

Table 3 shows the effect of minaprine, pargyline and combined pargyline–minaprine treatment on striatal HVA, DOPAC and 3-MT. Rats were injected with pargyline (100 mg kg^{-1} i.p.) 35 min before being killed and with minaprine (15 mg kg^{-1} i.p.) 30 min before death. Both drugs significantly reduced HVA and DOPAC concentrations and raised 3-MT. The changes in DA metabolites were greater after pargyline than after minaprine. After combined treatment with pargyline, minaprine, no longer affected the decrease in acid metabolites or the increase in 3-MT observed after MAO inhibition.

Discussion

The results show that minaprine lowers the concentrations of the acid metabolites of DA, DOPAC and HVA, an index of the intraneuronal metabolism (Di Giulio et al 1978). Minaprine appears not to act on the postsynaptic DA receptors since it does not displace [^{3}H]-spiperone from binding sites in-vitro.

These data, taken together, rule out the possibility that minaprine acts on the dopaminergic system by interacting directly in an agonistic or an antagonistic manner. Therefore the compound very probably acts indirectly. The increase of 3-MT caused by minaprine is not related to a release of DA because this effect usually causes an increase of all the DA catabolites (Algeri et al 1982; Ponzio et al 1981b). Furthermore, direct studies indicate that minaprine does not release [^{3}H]dopamine from striatal synaptosomes (T. Mennini, personal communication). An increase of 3-MT could also be caused

Table 2. Effect of minaprine (15 mg kg^{-1} i.p.) on concentrations of DA and its main catabolites in rat striatum at different intervals after administration.

	0	15′	30′	60′	90′	120′
DA	5514 ± 262	5921 ± 317	5727 ± 359	5849 ± 371	6094 ± 339	5870 ± 270
HVA	377 ± 30	283 ± 13*	190 ± 26**	222 ± 28**	231 ± 24**	247 ± 27**
DOPAC	655 ± 24	471 ± 13**	288 ± 14**	339 ± 57**	488 ± 21**	442 ± 31**
3-MT	23 ± 1	66 ± 5**	86 ± 8**	73 ± 14**	41 ± 3**	28 ± 3

Data are expressed as ng g^{-1} wet tissue (±s.e., n = 6).
*$P < 0.05$. $P < 0.01$ from controls by Dunnett's test.

Table 3. Effect of minaprine, pargyline and minaprine + pargyline on the concentration of DA, catabolites and 5HIAA in rat striatum.

	Controls	Minaprine	Pargyline	Pargyline+ Minaprine
3-MT	20 ± 0·7	70 ± 6**	189 ± 5**	192 ± 4***
DOPAC	521 ± 26	269 ± 11**	89 ± 4**	76 ± 6***
HVA	353 ± 13	222 ± 14**	147 ± 13**	145 ± 11***

Doses and treatment described in Methods.
Data are expressed as ng g^{-1} wet tissue (± s.e., n = 5).
*Different from controls $P < 0·05$ by Dunnett's test
** ,, ,, ,, $P < 0·01$,, ,, ,,
*** ,, minaprine $P < 0·05$,, ,, ,,

by blockers of DA uptake (Ponzio et al 1981b) although there are not enough selective inhibitors of DA uptake to establish whether such an effect is accompanied by the marked decrease of DOPAC and HVA induced by minaprine.

Finally, the effect of minaprine would be consistent with an inhibitory effect on monoamine oxidase (MAO). MAO inhibitors lower acid metabolites, raise 3-MT and in addition increase DA, an effect which is not fully shared by minaprine (see Table 2). With minaprine MAO inhibition must therefore be relatively mild and reversible.

In favour of this interpretation of the minaprine mechanism of action is the fact that in the striatum there is a decrease of another acid metabolite resulting from the action of MAO, viz 5-hydroxyindole acetic acid 5-HIAA (data not shown). In addition, P. Mandel (personal communication) observed that after in-vivo pretreatment with minaprine there is a reduction of MAO activity (substrates: 5-HT and tyramine) in the hypothalamus.

Finally, when minaprine was administered to rats with MAO completely inhibited by a massive dose of pargyline it no longer modified the increase in 3-MT induced by MAO inhibition. This suggests that minaprine acts by inhibiting MAO. If the increase in 3-MT were dependent on some mechanisms other than MAO inhibition such as enhanced release or uptake inhibition, we would expect the combined treatment with two drugs to have a greater effect than MAO inhibition alone. Such potentiation was in fact observed in the combined treatment with pargyline and a releaser such as nomifensine or methylphenidate (Algeri et al 1982), or a DA uptake inhibitor such as quipazine (Ponzio et al 1981b).

The final effect of minaprine on the DA system, judging from neurochemical studies, is probably a stimulant one due to increased availability of DA in the synaptic cleft as shown by the increase of 3-MT. DA agonists induce an increase of acetylcholine in the striatum, a finding also obtained after administration of minaprine (H. Ladinsky, personal communication).

All these considerations are made on the basis that minaprine itself is responsible for the observed effect on DA. However, that a metabolite may be involved to some extent cannot be excluded since minaprine is extensively metabolized (Davi et al 1981) with a half-life in the brain of about 15 min (Fong et al 1983).

In conclusion, minaprine appears to stimulate the dopaminergic system. Its action does not depend on a direct effect on DA postsynaptic receptors but it is caused by increased availability of DA.

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REFERENCES

Algeri, S., Ponzio, F., Achilli, G., Perego, C. (1982) Adv. Biochem. Psychopharmacol. 31: 219–228

Burt, D.-R., Creese, I., Snyder, S. H. (1977) Science 196: 326–328

Carlsson, A., Lindqvist, H. (1963) Acta Pharmacol. Toxicol. 20: 140–144

Davi, H., Dupont, P., Jeanniot, J. P., Roncucci, R., Cautreels, W. (1981) Xenobiotica 11: 735–747

Di Giulio, A. M., Groppetti, A., Cattabeni, F., Galli, C. L., Maggi, A., Algeri, S., Ponzio, F. (1978) Eur. J. Pharmacol. 52: 201–207

Fong, M. H., Abbiati, A., Benfenati, E., Caccia, S. (1983) J. Chromatogr. 259: 141–149

Garattini, S. (1981) Concours Medical suppl. no. 28: 28–30

Kehr, W. (1976) Naunyn Schmiedeberg's Arch. Pharmacol. 293: 209–215

Keller, R., Oke, A., I., Adams, R. N. (1976) Life Sci. 19: 995–1004

Kramer, C. Y. (1956) Biometrics 12: 307–310

Ponzio, F., Achilli, G., Algeri, S. (1981a) J. Neurochem. 36: 1361–1367

Ponzio, F., Achilli, G., Perego, C., Algeri, S. (1981b) Neurosci. Lett. 27: 61–67

Ponzio, F., Jonsson, G. (1978) Dev. Neurosci. 1: 80–89

Wermuth, C. G., Exinger, A. (1972) Aggressologie 13: 285–289