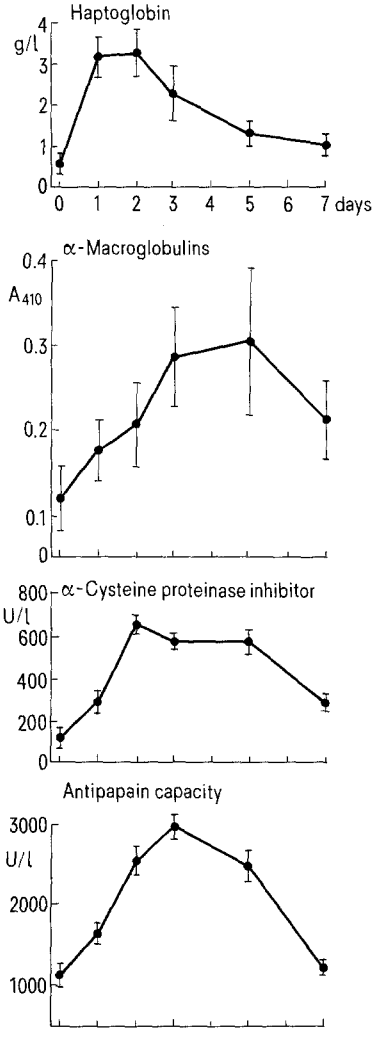
reaction with papain. In addition, the low mol.wt proteinase inhibitor found in rat serum inhibited papain, but the concentration of the inhibitor was very low[7].

The observed increase of α-CPI in the acute-phase rat plasma allows us to consider it as one of the markers of inflammation, more precisely as one of the acute-phase reactants with a positive response. Esnard and Gauthier[1] found that α-CPI isolated from acute-phase rat serum was very similar to α_1-acute phase globulin. Minakata et al.[13] established that pregnancy significantly enhanced the α-CPI level in human serum, while such pathological conditions as myoma of the uterus, endometritis, cervical cancer, ovarian cyst and ovarian cancer did not. Such results indicate that α-CPI is not an acute-phase reactant in humans. We also did not observe α-CPI level in sera of patients during polychemotherapy of ovarian cancer (not published).



Time-course changes in rat plasma levels of haptoglobin, α-macroglobulins, α-cysteine proteinase inhibitor and antipapain capacity following turpentine inflammation.

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Normetanephrine and metanephrine oxidized by both types of monoamine oxidase

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Summary. Both normetanephrine and metanephrine were found to be oxidized by both types of monoamine oxidase in mouse liver mitochondria. Both K_m and V_{max} values of type B MAO for both substrates were higher than those of type A MAO, which caused the shift of inhibition curves with clorgyline and deprenyl according to the increase in substrate concentration.
Key words. Mouse liver mitochondria; normetanephrine; metanephrine; monoamine oxidase.

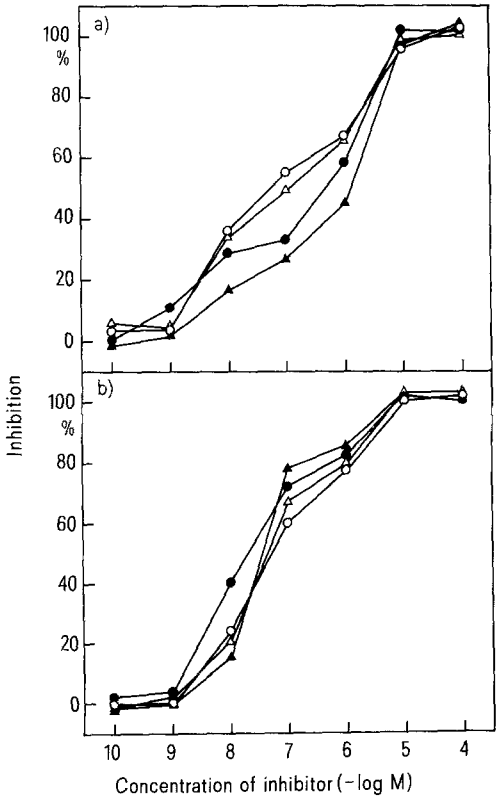
Normetanephrine and metanephrine are important O-methylated metabolites of norepinephrine and epinephrine, respectively, in mammalian tissues[1]. These compounds are further metabolized by monoamine oxidase (MAO) to yield finally 3-methoxy-4-hydroxymandelic acid. The enzyme responsible for the oxidation of normetanephrine and metanephrine has been believed to be type A MAO[2]. In the present communication, however, we demonstrate that these compounds are also oxidized by type B MAO in mouse liver mitochondria.

Materials and methods. Mitochondrial fraction was prepared

Apparent kinetic constants of type A and type B MAO in mouse liver mitochondria obtained by the pretreatment with clorgyline or deprenyl for normetanephrine and metanephrine as substrates*

Substrate	Deprenyl-treated** (type A-rich) MAO		Clorgyline-treated** (type B-rich) MAO	
	K_m (mM)	V_{max} (nmoles/mg protein/60 min)	K_m (mM)	V_{max} (nmoles/mg protein/60 min)
Normetanephrine	5.59	10.4	16.4	34.5
Metanephrine	4.00	9.80	8.26	28.6

* Each kinetic constant was determined graphically from Lineweaver-Burk plots with five substrate concentrations assayed in duplicate.
** The procedure for pretreatment is given in the text.



Inhibition of MAO in mouse liver mitochondria by clorgyline (a) and deprenyl (b) using different concentrations of normetanephrine and metanephrine as substrates. Keys: 0.5 mM normetanephrine (○); 2.0 mM normetanephrine (●); 0.5 mM metanephrine (△); 2.0 mM metanephrine (▲). Each point represents the mean obtained from duplicate determinations on a single enzyme source prepared form the pooled livers of 10 mice.

form the pooled livers of 20 male ddY mice weighing 20–35 g as described previously[3], and used for MAO assays. Clorgyline, a selective inhibitor of type A MAO[4], was generously supplied by May & Baker Ltd, Dagenham, England. Deprenyl, a selective inhibitor of type B MAO[5], was kindly donated by Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary.

MAO activities were measured fluorometrically by a modification[6] of the method of Guilbault et al.[7] and Snyder and Hendley[8]. For each assay (final volume, 0.6 ml), 0.28–1.52 mg of protein was used. The assays were carried out at pH 7.4 and 37 °C for 60 min. Under the conditions used, the assays were linear during incubation for 60 min.

For inhibition experiments with clorgyline and deprenyl, the assay mixtures were preincubated with each inhibitor at 37 °C for 20 min to ensure the maximal enzyme inhibition.

To obtain a single type A or type B activity, the mouse liver mitochondria were incubated with 10^{-6} M deprenyl or 10^{-6} M clorgyline at 37 °C for 20 min to inactivate type B or type A enzyme, respectively, and centrifuged at 18,000 × g for 10 min. The resulting pellet was suspended in 30 ml of 0.25 M sucrose in 0.01 M potassium phosphate buffer (pH 7.4) and recentrifuged at 18,000 × g for 10 min. This procedure was repeated once to remove free inhibitors. The resulting pellet was suspended in 5.0 ml of the above sucrose solution and used for kinetic analyses with the substrate concentrations of 0.625–10.0 mM.

Protein was measured by a modification[9] of the conventional biuret method.

Results. The figure shows the inhibition of MAO in mouse liver mitochondria by clorgyline and deprenyl, using two concentrations of normetanephrine and metanephrine as substrates. In all curves, shoulders appeared at about 10^{-7} M of clorgyline or deprenyl, showing that these substrates are deaminated by both types of MAO. The patterns for normetanephrine with both inhibitors are generally similar to those for metanephrine, but the normetanephrine deamination was slightly more sensitive to 10^{-7} or 10^{-6} M clorgyline, and slightly more resistant to 10^{-7} or 10^{-6} M deprenyl, showing a higher preference of normetanephrine towards type A MAO than that of metanephrine. By the increase in substrate concentration from 0.5 to 2.0 mM, the inhibition curves shifted appreciably; when judged at 10^{-7} M of the inhibitors, the sensitivity of MAO to clorgyline was decreased and that to deprenyl was increased by the increase in substrate concentration, showing an increased preference of both substrates towards type B MAO.

The table shows apparent Michaelis-Menten kinetic constants of both types of mouse liver mitochondrial MAO, obtained by their pretreatment with clorgyline or deprenyl, for normetanephrine and metanephrine as substrates. The K_m values of both types for both substrates were fairly high. Both K_m and V_{max} values of type B MAO for the substrates were higher than those of type A MAO, which accounts for the shift of the inhibition curves by the increase in substrate concentration shown in the figure.

Discussion. Important catecholamine metabolites normetanephrine and metanephrine have long been believed to be specific substrates for type A MAO[2]. However, in the present communication we have demonstrated that these substrates can also be oxidized by type B MAO in mouse liver mitochondria. We have also observed clear but small shoulders or plateaus in inhibition curves with normetanephrine and metanephrine using rat and human brain mitochondrial MAO (unpublished observation). However, the oxidation of these substrates by type B MAO was much more clearly demonstrated with mouse liver mitochondria, as shown in the present communication, because the ratio of type B to type A MAO in mouse liver is higher than that in rat or human brain[10].

Recently, well-known type A substrates, such as serotonin and norepinephrine, have been demonstrated to be oxidized also by type B MAO[10–12]; their nonspecificity is generally enhanced at high concentrations. This was also the case for normetanephrine and metanephrine (figure and table). It is, therefore, incorrect to draw a clear-cut conclusion that the so-called specific substrates are attacked exclusively by a single MAO type. The mode of in vivo metabolism of a biogenic monoamine by MAO seems to depend upon three factors, viz., the specificity of a monoamine itself for each type of MAO, the ratio of type A to type B MAO accessible to the monoamine, and monoamine concentration in the microenvironments of tissues.

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The dopamine autoreceptor agonist B-HT 920 markedly stimulates sexual behavior in male rats

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Summary. B-HT 920, a selective agonist at dopamine (DA) autoreceptors, strongly increased the incidence of penile erections (PE) in male rats, an effect which was dose-related and antagonized by haloperidol. B-HT 920 at 100 and 200 μg/kg i.p. significantly altered the copulatory pattern of sexually active male rats, reducing the number of mounts and intromissions as well as the latency to the first ejaculation, a stimulant effect which was confirmed in sluggish males at a dose of 100 μg/kg.
Key words. B-HT 920; dopamine autoreceptors; penile erections; sexual behavior; haloperidol; rat.

There is now considerable evidence to indicate a fundamental role of dopaminergic pathways in the regulation of sexual behavior in male rats. All dopamine (DA) receptor stimulants as yet tested elicit penile erections (PE) in isolated males[1–5] and influence the whole copulatory pattern, facilitating performance of the test in normal rats and restoring normal mating behavior in a percentage of impotent animals[6–8]. Although extrapolation from animal data to man always requires due caution, there does seem, to be agreement between animal results and clinical findings[9,10]. B-HT 920 is reported to be a specific agonist at DA autoreceptors[11,12]. It was on account of this selectivity of action, lacking in all other dopaminergic drugs as yet tested, which act at both D_1 and D_2 receptors[13], that we decided to study the influence of B-HT 920 on the sexual activity of male rats in the presence and in the absence of females in estrus.

Materials and methods. Animals and treatment. Male Wistar rats (Morini Farm, S. Polo d'Enza, Reggio Emilia, Italy) 230–250 g initial weight, were used. Female rats used as mating stimulus were of the same strain and weight. The animals were housed in cages 50 × 25 × 20 cm, eight animals per cage, with water and food freely available, at 22 ± 2 °C with a relative humidity of 60% and 12-h light/dark cycles (light on from 06.00 to 18.00 h). Tests were performed between 09.00 and 12.00 h, including the copulatory tests, as previous tests had established that there is no significant difference between performance under natural and reversed light conditions. 15 min before tests the animals were placed in glass observation cages to accustom them to the new environment. When animals were pretreated with the antagonist haloperidol, the drug was injected 45 min before B-HT 920. The substances were dissolved in distilled water and injected i.p. at a constant volume of 2 ml/kg. Doses of drugs refer to the weight of the salt. Controls were given the same volume of vehicle.

Evaluation of penile erections (PE) in male rats in absence of females. Immediately after the i.p. injection of B-HT 920 animals, in groups of 4 or 5 rats each, were observed continuously for 1 h by experienced researchers not aware of the animals' treatment. Each occurrence of PE was recorded for each animal responding during the observation period and the percentage of animals responding was calculated as well as the mean number ± SEM of PE per animal responding. The data for PE were analyzed by Student's t-test, with the level of significance set at $p < 0.05$.

Evaluation of male copulatory behavior. Female rats used as mating stimulus were brought into estrus with a s.c. injection of 0.12 mg estradiol benzoate 48–72 h before use. Male copulatory behavior was evaluated as by Dewsbury[14] and the following were recorded: mount and intromission latencies (ML and IL) (time elapsed from the introduction of the female into the cage until the 1st mount and intromission, respectively), mount and intromission frequency (MF and IF) (number of mounts and intromission preceding ejaculation), ejaculation latency (EL) (interval from the 1st intromission to ejaculation), postejaculatory interval (PEi) (time from the 1st ejaculation to the new 1st intromission). Tests were discontinued when IL or PEi were > 15 min or EL was > 30 min. Of a large number of male rats at the start of the experiments 22 were considered sexually active, five impotent and five sluggish, the remainder with no fixed pattern of behavior were discarded. Sexually active males were those which performed completely at least the last five preliminary mating tests out of the six conducted at 3-day intervals. Impotent males were those which never responded in any of the six preliminary mating tests. Sluggish males were those which, although showing a tendency to perform the test, failed to reach ejaculation in at least the last three out of the six preliminary tests.

The degree of constancy of copulatory behavior in the 5th and 6th pretests was checked with Student's t-test for paired data, which was also used to compare the values obtained before (mean of 5th and 6th tests) and after administration of B-HT 920 (level of significance set at $p < 0.05$), which was i.p. injected 15 min before the start of the final test.

Drugs. The following drugs were used. B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-4,5-d-azepine 2 HCl; Boehringer Ingelheim, Ingelheim am Rhein), haloperidol (Serenase, Lusofarmaco, Milan).

Results. Behavioral effects induced by B-HT 920 in males in absence of females. Consistently with previous results[5] control rats displayed PE in the 1-h observation period though the episodes were rare and restricted to a small percentage of animals (fig.). I.p. injection of B-HT 920 increased the percentage of animals responding, as well the mean number of PE per animal responding. The figure shows the impressive activity of