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STRAIN DIFFERENCES IN THE METABOLISM OF IMIPRAMINE BY RAT

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Abstract—The hyperthermic response to injected imipramine in reserpinized rats was compared in different strains of rats, such as Sprague–Dawley, Holtzman, Wistar and Long–Evans. Long–Evans rats are less reactive than all the other strains to the antireserpine activity of imipramine and show high levels of imipramine and low concentrations of desipramine (DMI) into the brain. *In vitro* experiments show that hepatic microsomal enzymes from Long–Evans rats metabolize imipramine to desipramine and also other substrates such as *p*.nitroanisol, aniline and aminopyrine to respective metabolites to a smaller extent than other rats. The reduced metabolic activity in addition to other possible reported effects, may be responsible for the reduced hyperthermic activity following imipramine in Long–Evans reserpinized rats.

IT WAS previously reported that the antireserpine activity exerted by imipramine depends from the species and the strains of the animals used.[1, 2] It was also suggested that imipramine would exert its anti-reserpine activity through the formation of a demethylated metabolite, namely desipramine.[1, 3, 4] Recently we have reported that the reversal of reserpine hypothermia induced by imipramine is more correlated to the desipramine than to the imipramine brain levels.[5]

The problem arises therefore whether the different antireserpine activity exerted by imipramine in various strains of rats may be the result of a different rate of formation of the active metabolite.

The studies hereafter reported show that the metabolic transformation of imipramine is not the only factor able to explain the different antireserpine activity of imipramine in various strains of rats.

MATERIALS AND METHODS

Female rats weighing 200 ± 10 g of the following strains were used: Sprague–Dawley (ALAL, Milan); Wistar (Allevamenti del Verbano, Paruzzaro (Novara); Long–Evans (Les Laboratoires Servier, Orléans, France); Holtzman (Willy Krispien, Hamburg, Germany).

The antireserpine activity was evaluated by measuring the increase in body temperature elicited by imipramine (20 mg/kg) or desipramine (15 mg/kg) injected intraperitoneally (i.p.) 16 hr after reserpine (5 mg/kg, i.v.). The experiments were performed at room temperature of 20° with 56 per cent of relative humidity. Desipramine and imipramine were determined in the whole brain according to Dingell, Sulser and Gillette.[2]

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Other hyperthermic reactions were evoked by different agents: 2,4 dinitrophenol (20 mg/kg, s.c.), reserpine (2·5 mg/kg, i.v.) and noradrenaline (4 μg/rat/min × 15 min, i.v.). Body temperature was continuously and automatically recorded.[6] These experiments were performed at a room temperature of 22°.

The metabolic activity of hepatic microsomal enzymes was measured on 9000 *g* supernatant fraction of livers obtained from 16 hr reserpinized rats (5 mg/kg, i.v.). The liver preparations were incubated with imipramine (1 μmole), *p*Nitroanisol (2 μmoles), aminopyrine (5 μmoles) or aniline (5 μmoles) and various cofactors as described by Kato and Takanaka.[7]

The incubated mixtures were analysed respectively for imipramine and desipramine,[2] *p*nitrophenol, 4 aminoantipyrine and *p*aminophenol[8]-content, after 30 min of incubation at 37°.

RESULTS

Table 1 shows that Long–Evans rats are less responsive than Sprague–Dawley, Wistar or Holtzman rats when imipramine was injected in hypothermic reserpinized animals and the hyperthermic response was measured.

TABLE 1. EFFECT OF IMIPRAMINE ON THE BODY TEMPERATURE OF RESERPINIZED RATS

No. rats	Rat strain	Body temperature (°C ± S.E.) at 0′	1 hr	2 hr	3 hr	Maximum increase Δ°C ± S.E.
11	Sprague–Dawley	29·5 ± 0·5	30·8 ± 0·7	32·0 ± 0·9	33·4 ± 1·1	+3·9 ± 0·7
5	Wistar	29·2 ± 0·7	31·4 ± 0·9	33·0 ± 0·9	34·2 ± 0·8	+5·0 ± 0·6
8	Long–Evans	30·9 ± 0·5	32·0 ± 0·7	32·5 ± 0·8	32·8 ± 0·9	+1·9 ± 0·5*
5	Holtzman	33·7 ± 1	35·8 ± 0·7	36·1 ± 0·5	36·7 ± 0·5	+3·0 ± 0·5

Room temperature was 20 ± 1°. Rats of the various strains weighed 200 g. Reserpine (5 mg/kg, i.v.) was given 16 hr before imipramine (20 mg/kg, i.p.).
* $P < 0·01$ in respect to Sprague–Dawley rats.

The results reported in Table 2 indicate that Long–Evans rats show always a desipramine brain concentration, following imipramine administration, lower than Sprague–Dawley rats, here considered the control strain as it is the one used in our laboratories. Imipramine disappears also more slowly from the brain of Long–Evans rats. However, when desipramine was injected in Long–Evans reserpinized rats the increase of body temperature was less marked than in Sprague–Dawley rats despite the fact that the levels of brain desipramine were higher in Long–Evans than in Sprague–Dawley rats (Table 3).

Also the hyperthermic effects elicited by an infusion of noradrenaline or an intravenous injection of reserpine are reduced in Long–Evans rats. In this strain of rats only 2,4 dinitrophenol fever attains a level comparable to that obtained with other strains (Table 4).

The *in vitro* experiments reported in Table 5 indicate that liver preparations from Long–Evans rats metabolize imipramine to form desipramine at a lower rate than the controls (Sprague–Dawley rats).

Holtzman and Wistar rats form more desipramine than Long–Evans rats but less than Sprague–Dawley strain.

TABLE 2. EFFECT OF IMIPRAMINE ON THE BODY TEMPERATURE OF RESERPINIZED RATS COMPARED TO THE BRAIN IMIPRAMINE AND DESIPRAMINE CONCENTRATIONS

	1 hr				2 hr				3 hr			
Rat strain	Body temp. (°C ± S.E.)	Δ°C	Imipramine (μg/brain ± S.E.)	DMI (μg/brain ± S.E.)	Body temp. (°C ± S.E.)	Δ°C	Imipramine (μg/brain ± S.E.)	DMI (μg/brain ± S.E.)	Body temp. (°C ± S.E.)	Δ°C	Imipramine (μg/brain ± S.E.)	DMI (μg/brain ± S.E.)
Sprague–Dawley	29·3 ±0·7 (9)	+0·7	20·6 ±0·8	3·0 ±0·4	32·1 ±0·9 (10)	+3·1	14·8 ±1·8	4·5 ±0·5	33·4 ±1·1 (11)	+3·9	11·0 ±0·9	5·9 ±0·7
Wistar	32·1 ±1·0 (6)	+1·5	19·7 ±1·1	3·5 ±0·8	33·3 ±0·7 (14)	+3·2	13·9 ±0·8	4·4 ±0·5	34·2 ±0·8 (5)	+5·0	11·6 ±0·5	4·9 ±0·6
Long–Evans	32·5 ±0·4 (8)	+1·5	24·9 ±2·9	1·7 ±0·2*	31·0 ±0·6 (8)	+1·4	24·6 ±1·7*	2·0 ±0·2*	32·8 ±0·9 (8)	+1·9*	20·3 ±2·1*	3·8 ±0·5*
Holtzman	34·5 ±1·1 (5)	±1·3	17·4 ±1·1	2·8 ±0·5	36·1 ±0·6 (6)	±2·6	12·7 ±1·0	5·2 ±0·3	36·7 ±0·5 (5)	±3·0	11·0 ±0·6	6·1 ±1·3

Imipramine (20 mg/kg, i.p.) was given 16 hr after reserpine (5 mg/kg, i.v.). The figures in brackets report the number of rats. Experiments were performed at 20° of room temperature.

* $P < 0{\cdot}01$ versus the group of Sprague–Dawley rats.

Δ°C is the difference between the body temperature at the time considered and the temperature in the same animals before administration of imipramine.

TABLE 3. EFFECT OF DESIPRAMINE (DMI) ON BODY TEMPERATURE OF RESERPINIZED RATS RELATED TO THE BRAIN DESIPRAMINE LEVELS

	1 hr			2 hr			3 hr		
Rat strain	Body temp. (°C ± S.E.)	Δ°C	DMI (μg/brain ± S.E.)	Body temp. (°C ± S.E.)	Δ°C	DMI (μg/brain ± S.E.)	Body temp. (°C ± S.E.)	Δ°C	DMI (μg/brain ± S.E.)
Sprague–Dawley	30·8 ±1·1	+1·7 (6)	9·0 ±0·7	32·9 ±0·8	+4·1 (10)	8·9 ±0·7	34·0 ±1·0	+5·5 (6)	8·6 ±1·0
Wistar	31·9 ±1·5	+2·5 (5)	8·6 ±1·4	34·6 ±0·5	+4·3 (13)	7·1 ±1·0	35·8 ±0·4	+5·3 (5)	7·0 ±0·7
Long–Evans	33·1 ±1·1	+1·6 (8)	11·8 ±0·7*	31·5 ±0·9	+1·9* (8)	8·9 ±1·1	34·2 ±0·8	+3·4* (8)	13·9 ±0·9*
Holtzman		—		36·5 ±0·3	+3·1 (6)	10·1 ±0·4		—	

DMI (15 mg/kg, i.p.) was given 16 hr after reserpine (5 mg/kg, i.v.). The figures in brackets report the number of rats.
* $P < 0{\cdot}01$ in respect to Sprague–Dawley rats.
Δ°C is the difference between the body temperature at the time considered and the temperature in the same animals before administration of desipramine.

TABLE 4. EFFECT OF VARIOUS HYPERTHERMIC AGENTS IN DIFFERENT STRAINS OF RATS

Rat strain	2·4 DNP (20 mg/kg, s.c.) Δ°C ± S.E.	* Noradrenaline (300 μg/kg, i.v.) Δ°C ± S.E.	Reserpine (2·5 mg/kg, i.v.) Δ°C ± S.E.
Sprague–Dawley	(17) 1·8 ± 0·1	(15) 1·0 ± 0·08	(3) 1·2 ± 0·17
Wistar	(12) 1·4 ± 0·2	(13) 0·7 ± 0·09	(9) 1·2 ± 0·2
Long–Evans	(5) 2·0 ± 0·3	(6) 0·3 ± 0·11	(5) 0·3 ± 0·1

The figures indicate the increase in body temperature after 2·4 dinitrophenol (2·4 DNP), noradrenaline and reserpine measured 60, 15 and 40 min respectively after administration (peak of the hyperthermic activity).

* Noradrenaline was administered by intravenous infusion (4 μg/rat/min × 15 min).

The figures in parentheses report the number of rats.

The rats given 2·4 DNP and noradrenaline weighed 200 g ± 10, the rats given reserpine weighed 150 g ± 10.

TABLE 5

No. rats	Rat strain	Imipramine unchanged μg ± S.E.	Imipramine unchanged % ± S.E.	Desipramine formed μg ± S.E.	Desipramine formed % ± S.E.
9	Sprague–Dawley	152 ± 9	48 ± 3	110 ± 6	35 ± 2
9	Wistar	158 ± 10	50 ± 3	84 ± 5*	26 ± 1*
14	Long–Evans	157 ± 9	50 ± 3	62 ± 5*	19 ± 2*
9	Holtzman	173 ± 7	55 ± 2	92 ± 5	29 ± 2*

The 9000 *g* liver homogenate supernatant fraction equivalent to 640 of liver was incubated with imipramine (1 μmole = 280 μg).

The reaction was stopped after 30 min.

Imipramine and desipramine were determined in the incubated mixture.

* $P < 0.01$ in comparison to Sprague–Dawley.

TABLE 6

Rat strain	pNO_2 Phenol (mμmoles/g/hr)	pNH_2 Phenol (mμmoles/g/hr)	4 Amino Antipyrine (mμmoles/g/hr)	Desipramine (mμmoles/g/hr)
Sprague–Dawley	(5) 618 ± 22	(5) 734 ± 41	(5) 343 ± 28	1154 ± 67
Long–Evans	(5) 263 ± 42*	(5) 374 ± 52*	(5) 120 ± 20*	620 ± 55†
Wistar	(5) 460 ± 52	(5) 721 ± 88	(5) 285 ± 28	869 ± 82†

The figures mean the amount of metabolites formed from pNO_2-anisol, aniline, aminopyrine and imipramine respectively, by a 9000 *g* supernatant fraction of liver homogenates (mμmoles/g/hr).

The figures in parentheses refer to the number of experiments.

* $P < 0.01$ versus the group of Sprague–Dawley rats.

† $P < 0.05$ versus the group of Sprague–Dawley rats.

In order to establish if the reported difference was specific for the metabolism of imipramine or common to other chemical substrates, which are metabolized through the liver microsomal enzymes, another experiment was performed.

Table 6 shows that *p*.nitroanisol, aniline and aminopyrine are transformed *in vitro* respectively into *p*.nitrophenol, *p*.aminophenol and 4-aminoantipyrine at a lower rate by the 9000 *g* liver fraction of Long–Evans than of Sprague–Dawley or Wistar rats.

DISCUSSION

The data reported in this paper indicate a different reactivity of the various strains of rats to the hyperthermic response elicited by imipramine in reserpinized animals. Long–Evans rats are the least responsive among the strains considered, namely Sprague–Dawley, Holtzman and Wistar. Since previous results suggested that imipramine must be transformed into desipramine to reverse the reserpine hypothermia,[5] measurements of brain desipramine were performed in the various strains of reserpinized rats after imipramine treatment.

The results obtained show that desipramine is present in the brain of all the strains studied.

These data, although at variance with the findings of Michaelis and Stille[9] in Holtzman rats, are in agreement with the results of several authors[2, 3, 10, 11] including Bickel and Weder[12] who found the presence of desipramine in similar concentrations in the brain of Holtzman and Wistar strains.

Long–Evans rats that are the least sensitive to the antireserpine activity of imipramine, show in the brain higher level of imipramine and a lower concentration of desipramine than the other strains, Sprague–Dawley, Holtzman and Wistar.

These data suggested the possibility of a different rate of *N*-demethylation in the liver of Long–Evans rats in respect to the other rat strains.

The *in vitro* experiments are in agreement with this hypothesis. They show also that Long–Evans rats possess a reduced liver microsomal enzymatic activity also for other substrates such as aniline, *p*.nitroanisol and aminopyrine.

However, in addition to the fact that desipramine accumulates at a lower rate it must be underlined that Long–Evans rats are less sensitive than other strains to the injected desipramine although high levels of brain desipramine are reached. That Long–Evans rats have a different sensitivity to drugs affecting body temperature is shown by the fact that they are not reactive when a hyperthermic response is evoked by adrenergic stimulation, directly by noradrenaline infusion or indirectly by intravenous reserpine.[14] However, Long–Evans rats can respond with a hyperthermic effect, similarly to other rat strains, when they are injected with 2,4 dinitrophenol, a known peripheral hyperpiretic agent.[13]

It may be therefore suggested that reserpinized Long–Evans rats show a low sensitivity to imipramine not only for a slow formation of desipramine but also because of a weak effect of the desipramine present in brain. Since desipramine increases body temperature through the adrenergic system[15] it is interesting that Long–Evans rats are less sensitive to the hyperthermic effect of injected or released catecholamines.

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REFERENCES

1. F. SULSER, M. H. BICKEL and B. B. BRODIE, *J. Pharmac. exp. Ther.* **144**, 321 (1964).
2. J. V. DINGELL, F. SULSER and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **143**, 14 (1964).
3. F. SULSER, J. WATTS and B. B. BRODIE, *Ann. N.Y. Acad. Sci.* **96**, 279 (1962).
4. M. H. BICKEL, *Prog. Drug Res.* **11**, 121 (1968).
5. A. JORI and D. BERNARDI, *J. Pharm. Pharmac.* **20**, 955 (1968).
6. A. JORI and S. PAGLIALUNGA, *Medna. Pharmac. exp.* **14**, 513 (1966).
7. R. KATO and A. TAKANAKA, *Jap. J. Pharmac.* **17**, 208 (1967).

8. D. GILBERT and L. GOLDBERG, *Food & Cosmet. Toxicol.* **3**, 417 (1965).
9. W. MICHAELIS and G. STILLE, *Life Sci.* **7**, 99 (1968).
10. B. HERRMANN, *Helv. phys. Acta* **21**, 402 (1963).
11. M. H. BICKEL and H. J. WEDER, *Archs int. Pharmacodyn. Thér.* **173**, 433 (1968).
12. M. H. BICKEL and H. J. WERDER, *Life Sci.* **7**, 1223 (1968).
13. A. BIANCHETTI, C. PUGLIATTI and A. JORI, *Medna. Pharmac. exp.* **17**, 401 (1967).
14. A. JORI, S. PAGLIALUNGA and S. GARATTINI, *Archs int. Pharmacodyn. Thér.* **165**, 384 (1967).
15. A. JORI, S. PAGLIALUNGA and S. GARATTINI, *J. Pharm. Pharmac.* **18**, 326 (1966).