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INFLUENCE OF A CHRONIC TREATMENT ON THE DISTRIBUTION OF AMITRYPTILINE AND METABOLITES IN RABBIT BRAIN

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Abstract—The distribution of amitryptiline and its basic metabolites in rabbit brain has been studied after acute and chronic administration of the drug.

The metabolizing activity of liver and brain homogenates from untreated and chronically pretreated rabbits has been also evaluated.

The results showed that after chronic pretreatment the disappearance of amitryptiline from brain areas is delayed and that the levels of drug metabolites are lower, while a marked increase in the metabolizing activity of liver and brain homogenates is noticed.

The results seem to suggest that chronic amitryptiline treatment induces membrane permeability changes, thus influencing the mechanism of transport and distribution of the same drug into and out of the cells.

THE METABOLIC pathways of amitryptiline, drug used in the therapeutic trials of depressed patients,[1–3] have been reported in various animal species.[4–6] Hucker and Porter[4,5] first studied *in vivo* and *in vitro* the metabolism of this drug in the rat.

Eschenhof and Rieder[6] studied the fate of amitryptiline in humans and its distribution in the rat.

Cassano *et al.*[7,8] found that amitryptiline, given intravenously, was highly concentrated in the central nervous system of mice and studied the distribution of this drug in several areas of cat brain using an autoradiographic technique.

The aim of the present study was to evaluate the possible influence of a chronic pretreatment with amitryptiline on the distribution of the same amitryptiline and its basic metabolites in rabbit brain.

For this reason we compared: (a) the distribution of amitryptiline and its basic metabolites after administration of a single acute dose of the drug in untreated animals with the distribution after administration of the same single dose in animals chronically pretreated. (b) the metabolizing activity of brain and liver homogenates from untreated and chronically pretreated rabbits.

MATERIALS

Animals

Male rabbits (65) of Fulvo di Borgogna strain (2·5–3 kg body weight) were used as experimental animals.

The animals were divided into two groups: (a) 45 rabbits for *in vivo* experiments; (b) 20 rabbits for *in vitro* experiments.

In vivo *experiments*

(1) "*Acute*" *treatment.* In the acute treatment 18 rabbits were treated with a single dose of 15 mg/kg of amitryptiline, administered intramuscularly.

The animals were killed by exsanguination 1·5, 4 and 6 hr after drug administration.

(2) " *Chronic*" *treatment.* In the chronic treatment 27 animals were treated for 40 days with a daily parenteral dose of 2·5 mg/kg of amitryptiline: tap water and food were given *ad lib.* during this period.

Twenty-four hours after the last drug administration the animals were divided into two groups.

The first group (18 rabbits) was treated intramuscularly with 15 mg/kg of the drug, and the animals were sacrificed after 1·5, 4 and 6 hr.

The second group (nine rabbits) was sacrificed at the same time intervals to evaluate the residual amounts of amitryptiline and its metabolites in the organs and the biological fluids (chronic blanks).

In vitro *experiments*

For the experiments *in vitro* two groups of 10 rabbits, one consisting of untreated animals, and the other of animals chronically treated for 40 days with a daily parenteral dose of 2·5 mg/kg of amitryptiline, were used.

METHODS

(a) *Experiments* in vivo

Brain, blood and kidney were immediately taken after sacrifice. The brain was dissected into cerebellum, brainstem and diencephalon, cerebral hemispheres. The urines were collected in the following way: (1) "Acute" animals, immediately after drug administration, were housed for 1·5, 4 or 6 hr in metabolic cages and their urines collected. After these time intervals the animals were sacrificed and the urine in their bladders was added to that eventually collected from the metabolic cages. (2) "Chronic" animals, 24 hr after the last chronic drug administration, were catheterized and their urines discarded; then, after the single drug administration (15 mg/kg), their urines were collected as described above. The cerebral tissues and the kidney were homogenized in 5 vol. of 0·01 HCl; the homogenates and the biological fluids were subjected to enzymatic hydrolysis, and then used for the chromatographic determination of amitryptiline and all its basic metabolites identified in rabbit in our previous works:[9–11] (10 hydroxy amitryptiline, 10–11 hydroxy amitryptiline, nortryptiline, 10 hydroxy nortryptiline, 10–11 hydroxy nortryptiline).

The basic metabolites of amitryptiline were evaluated in two groups: (1) the 10 and 10–11 hydroxy derivates of amitryptiline; (2) nortryptiline with its 10 and 10–11, hydroxy derivates.

Chromatographic experiments did not reveal between acutely and chronically treated rabbits qualitative differences in the nature of amitryptiline metabolites.

(b) *Experiments* in vitro

The experiments *in vitro* were performed on liver, brain *in toto*, white matter, gray matter, diencephalon, brainstem and the cerebellum from 10 untreated and 10 chronically treated animals.

The gray matter of the cerebral hemispheres was separated from the white matter using the slicing technique of McIlwain and Rodnight.[12]

Five animals from each group were used for determinations on liver and brain *in toto*, while the rest were used for the determinations on the separate brain areas.

The following procedure was adopted: samples were taken and homogenized at 800 rev/min (15 excursions/2 min) using a glass-teflon homogenizer with a clearance of 0·15 mm.

The medium was 5 vol. of cold 1·5%KCl for liver and 0·25 M sucrose for brain.

The homogenates were then centrifuged for 20 min at 1400 *g* at 0° in a refrigerated Sorvall centrifuge, and a volume of supernatant corresponding to 1 g of tissue was diluted with an equal volume of cold 0·1 M phosphate buffer, pH 7·4.

$NADP^+$ (2 mg) and G-6-P (20 mg) were then added to each sample and the mixture was warmed to 37°.

An aqueous solution of amitryptiline (100 μg) was added to each sample, and the samples were then incubated at 37° for 90 min in a Dubnoff thermostatic waterbath under air atmosphere.

The reaction was stopped by the addition of 0·1 ml glacial acetic acid.

The pH of the samples was adjusted to 4·9 and they were subjected to enzymatic hydrolysis followed by colorimetric determination of amitryptiline and metabolites.

RESULTS AND DISCUSSION

The first experiments at the level of central nervous system demonstrated the influence of a prolonged administration of amitryptiline on the distribution of the drug and metabolites in rabbit brain (Table 1).

In fact the cerebral areas in the chronic animals initially showed a lower concentration of amitryptiline than in the acute, but the rate of disappearance of the drug from the brain areas was significantly slower at the subsequent time intervals. The metabolites in the cerebral tissue of the chronic animals attained generally lower levels in all the observation time. Subsequently similar findings were confirmed in blood (Table 2) and in liver and lung of untreated and chronically treated rabbits. (Experiments still in progress.)

At the light of these findings, since we were unable to give a reasonable interpretation to these phenomena we thought useful to extend the investigation to the renal excretion of the drug and to the metabolizing activity of liver and brain homogenates.

In these experiments the levels of amitryptiline and metabolites in the kidney of the chronic animals were markedly lower than in the untreated nearly in all the observation time. By contrast the urinary levels of amitryptiline and metabolites were greatly higher in the chronic pretreated animals especially at the fourth and sixth hours (Table 2).

This reverse situation between kidney and urine means presently that the chronic administration of amitryptiline has in some way altered the renal mechanism of drug reabsorbtion. Now if we remember that some evidence has been reported in literature that chlorpromazine and tricyclic related drugs alter the permeability of biological membranes,[13–17] we can suggest that the chronic administration of amitryptiline could have hindered the reabsorption mechanism of the drug at the level of the renal epithelial cells membranes. With this in mind the delayed disappearance of amitryptiline from the brain areas of the chronically pretreated animals and the concomitant

Table 1. Concentrations* of amitryptiline and metabolites at various observation times in brain areas of untreated (A) and chronically pretreated (C) rabbits† after an i.m. administration of 15 mg/kg of amitryptiline

	A			C‡			Analysis of variance§		
								F	
	1·5 hr	4 hr	6 hr	1·5 hr	4 hr	6 hr	Treatment	Time	Interaction
Cerebral hemispheres									
Amitryptiline	266·44	72.82	36·70	180·58	72·28	49·76	5·73‖	119·60¶	9·19¶
10 OH Amitryptiline 10–11 OH Amitryptiline	25·00	11·72	6·04	9·26	7·32	3·10	33·89¶	30·56¶	9·37¶
10 OH Nortryptiline 10–11 OH Nortryptiline Nortryptiline	11·00	6·70	3·10	3·36	1·40	1·32	54·10¶	18·92¶	6·52¶
Brainstem and diencephalon									
Amitryptiline	269·18	71·34	33·52	170·04	74·48	48·24	6·16‖	101·54¶	10·98¶
10 OH Amitryptiline 10–11 OH Amitryptiline	33·46	28.00	15·28	13·32	7·24	4·50	151·16¶	31·16¶	5·31‖
10 OH Nortryptiline 10–11 OH Nortryptiline Nortryptiline	29·48	15·72	9·50	7·20	5·00	2·90	53·87¶	15·69¶	6·81¶
Cerebellum									
Amitryptiline	260·14	79·28	45·58	162·60	78·90	50·80	9·15¶	95·96¶	10·66¶
10 OH Amitryptiline 10–11 OH Amitryptiline	40·08	35·14	24·98	15·26	14·14	9·54	201·27¶	18·45¶	3·58‖
10 OH Nortryptiline 10–11 OH Nortryptiline Nortryptiline	38·36	19·68	13·32	10·90	8·78	3·10	63·43¶	22·26¶	7·69¶

* Data expressed as micrograms × 10 g of tissue are means of five determinations on five animals.

† The animals were pretreated for 40 days with a daily i.m. dose of 2·5 mg/kg of amitryptiline.

‡ The concentrations of the chronic animals have been subtracted with the concentrations of the chronic blanks which 24 hr after last chronic drug administration ranged from 0·5 to 1 μg × 10 g of tissue.

§ The statistical evaluation was performed with the analysis of the variance with two criteria (treatment and time) with interaction.

‖ F significant at 5 per cent level.

¶ F significant at 1 per cent level.

TABLE 2. CONCENTRATIONS* OF AMITRYPTILINE AND METABOLITES IN KIDNEY, URINE, BLOOD OF UNTREATED (A) AND CHRONICALLY PRETREATED (C) RABBITS† AFTER AN i.m. ADMINISTRATION OF 15 mg/kg OF AMITRYPTILINE

	A			C‡			Analysis of variance§		
								F	
	1·5 hr	4 hr	6 hr	1·5 hr	4 hr	6 hr	Treatment	Time	Interaction
Kidney									
Amitryptiline	539·60	161·60	41·82	110·96	74·42	51·62	18·19¶	17·85¶	11·30¶
10 OH Amitryptiline 10–11 OH Amitryptiline	21·40	16·12	12·96	8·12	6·50	4·58	145·87¶	16·17¶	2·90¶
10 OH Nortryptiline 10–11 OH Nortryptiline Nortryptiline	4·5 ± 0·9	1·9 ± 0·2	—	3·9 ± 0·1	1·1 ± 0·2	—			
Urine**									
Amitryptiline	15·20	54·34	253·50	22·30	320·66	942·80	28·62¶	32·91¶	10·99¶
10 OH Amitryptiline 10–11 OH Amitryptiline	9·64	26·88	44·48	17·42	177·82	561·04	25·36¶	14·65¶	11·48¶
10 OH Nortryptiline 10–11 OH Nortryptiline Nortryptiline	5·72	10·86	15·88	10·92	20·16	124·52	15·83¶	14·45¶	11·09¶
Blood									
Amitryptiline	5·44	4·56	1·30	4·00	2·40	1·20	27·97¶	75·81¶	6·70¶
10 OH Amitryptiline 10–11 OH Amitryptiline	2·6 ± 0·15	1·5 ± 0·18	0·52 ± 0·11	0·75 ± 0·18	0·45 ± 0·15	traces			
10 OH Nortryptiline 10–11 OH Nortryptiline Nortryptiline	0·42 ± 0·07	0·42 ± 0·085	0·35 ± 0·06	traces	traces	0·22 ± 0·06			

* Data expressed as micrograms × 10 g of tissue are means of five determinations on five animals.

† The animals were pretreated for 40 days with a daily i.m. dose of 2·5 mg/kg of amitryptiline.

‡ The concentrations of the chronic animals have been subtracted with the concentrations of the chronic blanks which 24 hr after last chronic drug administration ranged from 0·5 to 1 μg × 10 g of tissue.

§ The statistical evaluation was performed with the analysis of the variance with two criteria (treatment and time) with interaction.

‖ F significant at 5 per cent level.

¶ F significant at 1 per cent level.

** Data refer to the total amounts of drug and metabolites present in collected urines.

Table 3. Amounts of metabolized amitryptiline* from liver, brain *in toto*, cerebral areas homogenates of untreated (A) and chronically pretreated (C) rabbits†

Tissue		Amitryptiline	10 OH Amitryptiline 10–11 OH Amitryptiline	Nortryptiline 10 OH Nortryptiline 10–11 OH Nortryptiline	Recoveries (%)
Liver	A	43·1 ± 0·79	36·8 ± 0·50	9·1 ± 0·19	88·9
	C	27·9 ± 0·61‡	44·8 ± 1·13‡	17·1 ± 0·33‡	89·8
Brain *in toto*	A	83·5 ± 1·18	3·4 ± 0·17	1·0 ± 0.06	87·9
	C	77·8 ± 1·00‡	7·2 ± 0·26‡	3·4 ± 0·15‡	88·5
White matter	A	81·0 ± 1·29	1·0 ± 0·05	0·5 ± 0·04	82·6
	C	79·7 ± 0·91§	3·9 ± 0·10‡	0·8 ± 0·06‡	84·5
Gray matter	A	80·3 ± 1·53	4·8 ± 0·18	1·4 ± 0·07	86·4
	C	68·8 ± 1·16‡	9·1 ± 0·30‡	5·6 ± 0·17‡	83·5
Brainstem	A	81·6 ± 1·06	1·0 ± 0·04	0·7 ± 0·04	83·3
	C	79·5 ± 1·50§	1·2 ± 0·08§	0·8 ± 0·07§	81·5
Diencephalon	A	79·2 ± 1·31	4·9 ± 0·16	1·0 ± 0·04	85·1
	C	72.4 ± 0.99‡	8·2 ± 0·18‡	1·3 ± 0·07‡	81·9
Cerebellum	A	73·6 ± 0·82	3·4 ± 0·10	1·3 ± 0·09	78·3
	C	62·9 ± 0·97‡	10·5 ± 0·23‡	9·9 ± 0·19‡	83·4

Results are expressed as micrograms/g/90 min, as a mean ± S.E. of five animals.
* The experiments were performed adding 100 μg of amitryptiline to the incubation mixture (see Methods).
† The animals were pretreated for 40 days with a daily i.m. dose of 2·5 mg/kg of amitryptiline.
‡ Statistically highly significant difference, as compared with the values of the untreated rabbits ($P<0·01$).
§ No significant difference ($P>0·05$).

diminution of metabolites might be similarly interpreted by an amitryptiline influence on the transport of the drug through the cells membranes of the nervous tissue.

Thus the low concentration of metabolites in the brain areas of the chronic animals might therefore be considered the result of an amitryptiline membrane effect slowing or preventing drug uptake by enzymes rather than an amitryptiline inhibitory effect on the enzymatic metabolizing activity.

This view is supported by the results of our *in vitro* experiments which showed for liver homogenates from chronically treated animals an increase in the metabolized amounts of amitryptiline of about 15 per cent and for cerebral areas an increase ranging from 1·0 to 18 per cent in comparison to the metabolized amounts by untreated animals (Table 3).

In conclusion the present results although still incomplete, seem to suggest that membrane permeability changes induced by the chronic treatment could have determined the different distribution pattern of amitryptiline and metabolites in the brain areas and emphasize the importance of the effects of a chronic treatment on drug metabolism and distribution.

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