Acceleration of the Cerebral Dopamine Turnover by Chlorpromazine

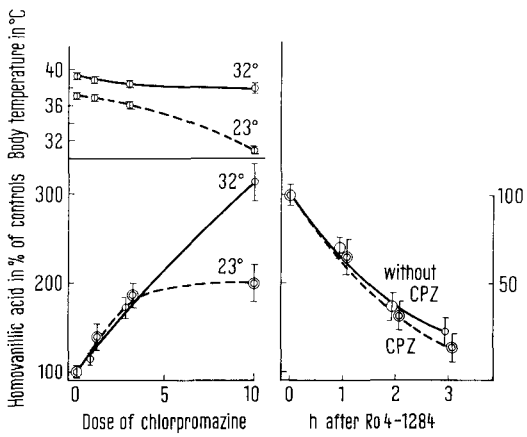
Neuroleptics, e.g. chlorpromazine (= 2-chloro-10-(3-dimethylaminopropyl)-phenothiazine), have been reported to markedly increase the endogenous homovanillic acid (HVA) of the brain stem of cats and rabbits, thereby decreasing not at all or only moderately the cerebral dopamine content[1-3]. Furthermore, chlorpromazine and haloperidol enhance the accumulation of cerebral 3-methoxytyramine induced by pretreatment with monoamine oxidase (MAO) inhibitors without influencing the dopamine increase[4]. These findings lead to the assumption that neuroleptics might possibly enhance the dopamine synthesis by a feed-back mechanism in consequence of a blockade of dopaminergic receptors[1,4].

The above hypothesis needs, however, further corroboration, since other possible modes of action of the neuroleptics are not to be excluded. Therefore, investigations on the mechanism of action of chlorpromazine will be reported in the present paper.

Experimental. Albino rats (60–90 g) and guinea-pigs (250–300 g), both from a closed colony (breed of Fullinsdorf), have been used after a fasting period of 16 h. In most of the experiments, the rats were kept at an environmental temperature of 32 °C in order to avoid hypothermia by chlorpromazine. Chlorpromazine was injected either alone (once or repeatedly) or 3/4 h after a short-acting monoamine releaser (benzoquinolizine derivative Ro 4-1284[5]). The latter compound causes a temporary increase of the endogenous cerebral HVA, which is probably due to the metabolism of released dopamine and which reaches its maximum after about 3/4 h. In other experiments, chlorpromazine was administered 2 and 16 h after the MAO inhibitors pargyline and iproniazid respectively. Determinations of HVA, 5-hydroxyindoleacetic acid (5-HIAA) and dopamine in the brain stem (including the basal ganglia) were carried out spectrophotofluorimetrically[6-9].

Results. (1) Chlorpromazine causes a marked dose-dependent increase of the endogenous HVA content in the brain stem of rats and guinea-pigs. In rats, the rise is maximal about 3 h after 10 mg/kg of the drug; normal levels are re-established after about 16 h. With 1 and 3 mg/kg chlorpromazine, this effect is similar at room temperature (23 °C) and at an environmental temperature of 32 °C. With 10 mg/kg chlorpromazine, the HVA increase is significantly higher at 32 °C (no drop of body temperature) than at 23 °C (marked drop of body temperature) (Figure). (2) Chlorpromazine induces no significant change in the levels of endogenous dopamine and 5-HIAA in the brain stem of rats (Table). (3) Chlorpromazine does not counteract the decrease of the cerebral HVA levels which have been elevated by previous administration of the monoamine releaser Ro 4-1284 (Figure). (4) After inhibition of MAO by iproniazid and pargyline, which markedly diminishes the endogenous HVA in the brain stem[6], chlorpromazine no longer causes a HVA increase (Table).

Discussion. The results in rats kept at elevated environmental temperature support earlier findings with rabbits, according to which the chlorpromazine-induced



Effect of chlorpromazine on normal and elevated levels of homovanillic acid (HVA) in brain stem of rats. Left: Increase of HVA and decrease of rectal temperature in animals kept at an environmental temperature of 23 or 32 °C. The animals were sacrificed 3 h after i.p. injection of the drug. Absolute levels of HVA of controls: 0.12 ± 0.01 µg/g (= 100%). Each point represents an average of 5 experiments ± S.E. Right: Influence of chlorpromazine (CPZ) on the decrease of endogenous HVA levels elevated by previous i.p. administration of 25 mg/kg Ro 4-1284. 10 mg/kg chlorpromazine were injected i.p. 3/4 h after Ro 4-1284 at the time of the maximal HVA elevation (= 0 time). Absolute increase of HVA (from levels in untreated animals) at 0 time = 0.25 ± 0.015 γ/g (= 100% = controls). Each point represents an average of 4–6 experiments ± S.E. Experiments carried out at environmental temperatures of 23 and 32 °C were taken together since the results were identical.

Effect of chlorpromazine i.p. on the metabolism of homovanillic acid (HVA), dopamine, and 5-hydroxyindoleacetic acid (5-HIAA) in the brain stem of rats

Iproniazid was administered 16 h and pargyline 2 h before chlorpromazine. The animals were sacrificed 2 h after chlorpromazine and 4 or 18 h after pargyline and iproniazid respectively. Each result represents an average of 3–4 experiments ± S.E. and is expressed in % of untreated controls (= 100%). The animals were kept at an environmental temperature of + 32 °C.

Drug	Dose (mg/kg, i.p.)	HVA	Dopamine	5-HIAA
Chlorpromazine	10	246 ± 15	106 ± 1	113 ± 5[a]
Chlorpromazine	8 · 10[b]		103 ± 2	
Iproniazid	100	15 ± 3		
Pargyline	100	15 ± 3		
Iproniazid + Chlorpromazine	100 10	8 ± 0		
Pargyline + Chlorpromazine	100 10	8 ± 3		

[a] 20 mg/kg chlorpromazine i.p. [b] 2 · 10 mg/kg daily with an interval of 7 h for 4 days; sacrificed 2 h after the last chlorpromazine injection.

[1] N. E. Andén, B.-E. Roos, and B. Werdinius, Life Sci. *3*, 149 (1964).
[2] R. Laverty and D. F. Sharman, Br. J. Pharmacol. *24*, 759 (1965).
[3] B.-E. Roos, J. Pharmac. (Lond.) *17*, 820 (1965).
[4] A. Carlsson and M. Lindqvist, Acta pharmac. (Kbh.) *20*, 140 (1963).
[5] A. Pletscher, A. Brossi, and K. F. Gey, Int. Rev. Neurobiol. *4*, 275 (1962).
[6] N. E. Andén, B.-E. Roos, and B. Werdinius, Life Sci. *2*, 448 (1963).
[7] D. F. Sharman and E. Smith, J. Neurochem. *9*, 403 (1962).
[8] A. Pletscher, W. P. Burkard, and K. F. Gey, Biochem. Pharmac. *13*, 385 (1964).
[9] A. Carlsson and B. Waldeck, Acta physiol. scand. *44*, 293 (1958).

increase of the HVA content in the brain stem is not due to hypothermia but rather to a direct action of the drug[1]. This direct effect may be diminished by hypothermia, as seen in the experiments carried out with 10 mg/kg chlorpromazine in different environmental conditions (Figure). The following possible mechanisms of action of chlorpromazine in inducing an increase of cerebral HVA can probably be excluded: (a) Release of dopamine: The endogenous dopamine of the brain stem does not decrease even after repeated administration of chlorpromazine. (b) Diminution of the HVA elimination from the brain: Chlorpromazine does not counteract the decrease of the endogenous HVA in the brain stem which had been elevated by previous administration of the monoamine releaser Ro 4-1284. Furthermore, in rats, as previously reported for rabbits and cats[1,2], chlorpromazine causes no increase of cerebral 5-HIAA, which might be expected if the drug had an unspecific effect on the outflow of HVA. (c) Acceleration of dihydroxyphenylalanine-dihydroxyphenylpyruvic acid transaminase, which might yield HVA without intermediary formation of dopamine: The MAO inhibitors pargyline and iproniazid abolish the chlorpromazine-induced HVA increase although the drugs do not seem to interfere with the transaminase[10]. This indicates that, in the experiments with chlorpromazine alone, the HVA rise might be due to an increased oxidative deamination of dopamine.

The above findings support the hypothesis that the chlorpromazine-induced increase of the cerebral HVA level is caused by an accelerated turnover of dopamine, possibly due to a compensatory enhancement of the dopamine synthesis in consequence of a blockade of dopaminergic receptors in the extrapyramidal centres. Experiments with various other psychotropic drugs are in agreement with this assumption. Accordingly, only neuroleptics which may impair the function of the extrapyramidal centres (e.g. phenothiazines and butyrophenones) increase the cerebral HVA, whereas drugs which interfere relatively little with the extrapyramidal system (thymoleptics, tranquilizers, hypnotics) do not change the HVA level in the brain stem[11].

Zusammenfassung. Im Stammhirn von normothermen Ratten bewirkt Chlorpromazin einen Anstieg von Homovanillinsäure (HVS), der bei Hypothermie verringert wird. Dieser Anstieg geht nicht mit einer Verminderung der Dopaminkonzentration einher und bleibt nach Hemmung der Monoaminoxydase aus. Die durch Vorbehandlung mit einem kurzwirkenden Monoaminfreisetzer (Benzochinolizinderivat Ro 4-1284) erhöhte endogene HVS zeigt nach Chlorpromazin keinen verzögerten Abfall.

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[10] K. F. GEY, in preparation.
[11] M. DA PRADA and A. PLETSCHER, in preparation.

Gonadotrophic Control of Lamina propria and Laminar Collagen of Testis

It has been observed earlier that testicular tubules of toad (*Bufo melanostictus*), during cold torpor, contain huge amounts of sudanophil lipids[1]. Further investigation has indicated these lipids to be ketosteroids and of a similar nature to those found in interstitial cells (unpublished). It is postulated on the basis of the following experiments that these lipids, accumulated in interstitial cells, have pierced through the tubular wall into the tubules, which have been considerably weakened due to the probable absence of gonadotrophin from the pituitary, causing atrophic changes in the testis during the torpid state[2].

Toads, in torpid state, acquired during cold weather (January) were divided into two groups of 24 each, with an average body weight of around 30 g. One group received 25 I.U.[3] of PMS gonadotrophin[4] per animal/day for 7–10 days parenterally through the dorsal lymph sac[5]. After the experiment, testes from each group of animals were fixed in 4% formol, and after the usual paraffin procedure and sectioning they were stained by the PAS-allochrome technique[6] for collagen and lamina propria and compared with those of normal active toads at breeding time.

Figure 1 shows a thin branching disintegrated lamina of testis during torpid state with hardly any trace of collagen around it. String-like elastic recoiled lamina probably indicates the tearing away at high tension. On

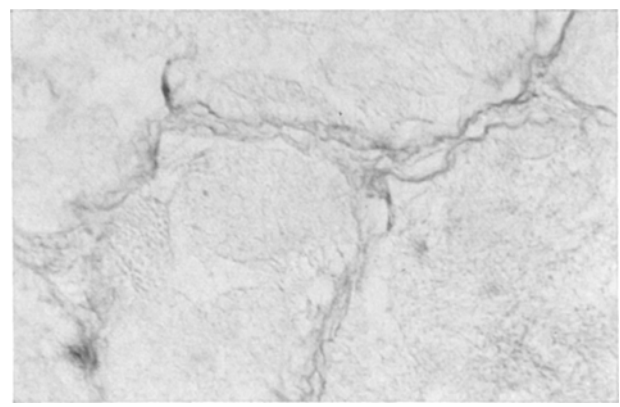

Fig. 1. Toad's testis (cold torpor). Note disintegrating torn lamina and absence of collagenous support. ×192.

[1] M. MUKHERJI, J. Histochem. Cytochem. *8*, 242 (1960).
[2] C. P. LYMAN and P. O. CHATFIELD, Physiol. Rev. *35*, 403 (1955).
[3] J. S. WANNAN, Med. J. Australia *2*, 83 (1952).
[4] Organon Lab. Ltd.
[5] J. R. VALLE, J. C. PENHOS, and B. A. HOUSSAY, Rev. Soc. Argent. Biol. *28*, 1 (1952).
[6] R. D. LILLIE, Am. J. clin. Path. *24*, 484 (1951).