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COMPARATIVE STUDY ON THE ACTIVITY OF CHLORPROMAZINE AND 7-HYDROXYCHLORPROMAZINE IN THE ISOLATED PERFUSED RAT BRAIN

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Abstract—Chlorpromazine (CPZ), 7-hydroxychlorpromazine (7-OH-CPZ), 8-hydroxychlorpromazine, 3,7-dihydroxychlorpromazine, and chlorpromazine sulfoxide were investigated. Isolated rat brains were perfused for 30 min with 100 ml of a perfusion medium containing approx 30 per cent bovine red cells (v/v), 2 g bovine serum albumin, 14 mM glucose and one of the phenothiazines in a concentration of 5–100 μM (10 μM for comparative studies). The main dopamine metabolite, homovanillic acid (HVA), was measured fluorimetrically in the striatum of the isolated brain. The EEG was recorded with two symmetrical bipolar leads from the parietal regions at various times and was stored on a magnetic tape. The recordings were evaluated visually and quantitatively by automatic analysis. CPZ and 7-OH-CPZ changed the EEG and increased the striatal HVA level significantly but CPZ seemed to be more active. The other phenothiazines studied did not produce clear effects. The increase of the HVA level in the striatum was correlated with the increase of the EEG amplitude and of the percentages of theta and delta waves as well as with the decrease in the percentage of beta waves.

The biotransformations of chlorpromazine (CPZ) result in a prodigious number of metabolites. Both the CPZ nucleus and the dimethylaminopropyl side chain undergo substantial metabolic pathway. 7-Hydroxychlorpromazine (7-OH-CPZ) is one of the important metabolites as it has been demonstrated to reach blood levels in man comparable to those of the parent drug CPZ and it seems to be a better predictor of clinical response than the levels of the parent drug [1]. Furthermore, Creese *et al.* [2] have reported a considerable affinity of 7-OH-CPZ to the dopamine receptor.

However, it is difficult to compare exactly the central activity of CPZ in intact animals with that of its metabolites because CPZ as well as its metabolites are further transformed predominantly in the liver. Therefore, it is an intriguing possibility to use the isolated perfused rat brain for studying the central activity of the phenothiazines. In previous work we have already found chlorpromazine-*N*-oxide to be at least as active as CPZ in the isolated rat brain [3]. The purpose of this study was to compare the effects of CPZ and particularly 7-OH-CPZ on the EEG and on the dopamine metabolism of the isolated perfused rat brain.

MATERIALS AND METHODS

Materials. Bovine serum albumin (quality: dry, pure) was purchased from Behring-Werke (Marburg), and homovanillic acid from Roth (Karlsruhe). The drugs used in this study were chlorpromazine = CPZ (Bayer, Leverkusen), chlorpromazine sulfoxide = CPZ-SO (Rhône-Poulenc, Paris), 7-hydroxychlorpromazine = 7-OH-CPZ (I.S. Forrest, Veterans Administration Hospital, Palo Alto, U.S.A.), 8-hydroxychlorpromazine = 8-OH-CPZ, 3,7-dihydroxychlorpromazine = 3,7-di-OH-CPZ (A.A. Manian, N.I.H., Bethesda, U.S.A.). All other chemicals were of reagent or ultrapure grade. Male Sprague-Dawley rats weighing 180–250 g were used. The animals had free access to food (standard diet Altromin®) and tap water until they were used.

Preparation of the isolated perfused rat brain. Preparation of the isolated rat brain [4] was performed under urethane anesthesia (1.2 g/kg, i.p.). A closed-circuit perfusion with 100 ml of a simplified blood was carried out under subdued light as standardized in our laboratory [5,6] using apparatus I described by Fleck *et al.* [7]. Arterial pressure was maintained between 100 and 120 mg/Hg, as monitored with a pressure transducer. The perfusion rate was 3–5 ml/min. At the end of the perfusion period of 30 min the brains were quickly removed. The striata were separated as described by Glowinski and Iversen [9] and were stored in liquid nitrogen until further analysis.

Artificial blood. The artificial blood used for perfusion consisted of washed bovine red cells, bovine serum albumin, and Krebs–Henseleit solution to give a final haematocrit value of about 30%, an albumin concentration of 2 g % and a glucose concentration of 14 mM. One of the drugs was added to the simplified blood to give a final concentration of 10 μM. In control experiments no drug was added.

Recording of the EEG. Four electrodes were placed as two symmetrical bipolar leads on the parietal regions [8]. The EEG could be watched con-

tinuously on an oscilloscope and was recorded at various times on paper and on magnetic tape. The tape recordings were made 10, 20 and 30 min after the beginning of perfusion for a duration of 4 min for each recording period.

Determination of homovanillic acid (= HVA). The corpus striatum was homogenized in 4.0 ml of ice cold 0.4 N perchloric acid in a 5 ml glass homogenizer (Potter–Elvehjem). HVA was separated by small Sephadex-G 10 columns and was measured fluorimetrically according to Anden *et al.* [10]. The recovery of HVA was tested by adding amounts of the authentic compound ranging from 50 to 250 ng to tissue homogenates. The mean recovery was 70.2 ± 2.4% (mean ± S.E.M.) in eight tests.

Thin layer chromatography (TLC). In order to rule out metabolism of the phenothiazines tested, a small amount of the albumin solution obtained by centrifugation of the simplified blood was examined by TLC. The albumin solution was extracted twice with dichlormethane/*n*-propanol (7 + 3). The combined extracts were evaporated and the residue was chromatographically analysed (TLC plate: 20 × 20 cm, 500 μm thick, Merck Silica Gel F 254; solvent system: methanol–acetone–NH_3, 25%, 50:50:1). The spots were observed under u.v. light.

Evaluation of the EEG. The EEG stored on magnetic tape was reproduced on paper for visual evaluation. For quantitative estimation of amplitude and frequency properties the biosignal apparatus Nicolet 1070 N was used with plug-ins for amplitude and interval histography.

For the evaluation of the *amplitude* we used the method described by Saunders [11]. After analog/digital conversion, the EEG amplitudes were ranged automatically in 25 classes. Measurements were performed with a sampling frequency of 5 kHz and were stopped after 2.10^5 measures. A standard amplitude was calculated, i.e. standard deviation of amplitudes with respect to the average of the EEG which equals 0 μV.

The *frequency* composition of the EEG was estimated by interval histography [12]. By this method the duration of the time interval between successive baseline-crossings of the EEG was measured automatically. The observed intervals were ranged in 256 interval classes between 4 and 1024 msec. The data processing stopped automatically when 5000 intervals were stored in the memory. The time resolution used was 4 msec. The resulting distribution (interval histogram) was related to the frequency content of the EEG. The frequency spectrum of the histogram was subdivided into four frequency bands:

beta: 13.2–32 c/sec
alpha: 7.6–13.2 c/sec
theta: 4–7.6 c/sec
delta: 0.98–4 c/sec

Statistical analysis. The data obtained from amplitude and interval histography were examined statistically using the Kruskal and Wallis test [13] in order to discover any global differences between the experimental series. When this test was proved significant, multiple bilateral comparisons between each group were performed by means of the Nemenyi test [13]. In order to detect any correlations between the EEG data and the striatal HVA level, rank correlations according to Spearman [13] were calculated. The HVA values (Table 3) were analysed by the U-test [13].

RESULTS

Visual evaluation of the EEG

The visual evaluation of the EEG of the isolated perfused rat brain revealed a dominant beta activity in control experiments. Isolated spikes rarely appeared (Fig. 1).

CPZ added to the simplified blood produced dose-dependent EEG effects (Fig. 1). Whereas 5 μM CPZ did not clearly change the EEG, distinct effects were seen after 10 μM CPZ. The standard amplitude as well as the percentage of theta and delta waves increased. Isolated or grouped spikes or spike and wave phenomena frequently appeared. These EEG modifications were still more pronounced with 50 μM CPZ. When CPZ concentration was raised to 100 μM, intermittent low voltage periods and bursts of spikes, sharp and slow waves could be observed in the EEG. In preliminary experiments the effects of the hydroxylated CPZ metabolites and CPZ-SO on the EEG of the isolated rat brain were examined. No substantial changes were found after administration of 3,7-diOH-CPZ, 8-OH-CPZ and CPZ-SO (10 μM in each experiment), whereas 10 μM 7-OH-CPZ altered the EEG significantly. Isolated and grouped spikes and high-voltage sharp waves similar to the CPZ effects appeared in the EEG. Therefore, more extensive experimental series were performed with CPZ and 7-OH-CPZ as well as CPZ-SO which is probably an uneffective metabolite (Fig. 2).

Quantitative EEG analysis

The interval histography confirmed the visual impression that the fast waves in the beta range predominated in the EEG of the isolated perfused

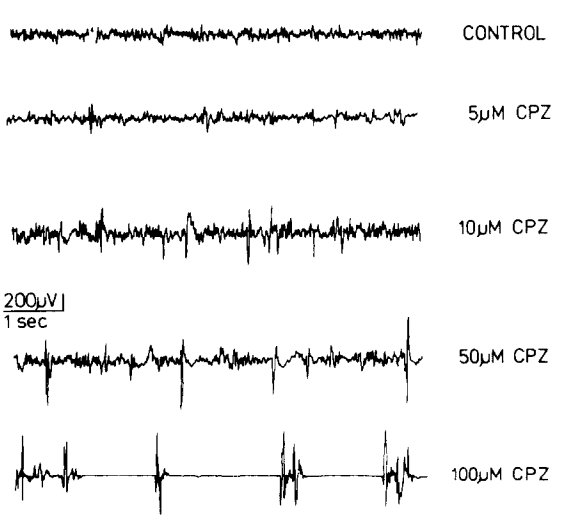

Fig. 1. Dose-dependent EEG effects of CPZ in the isolated perfused rat brain. EEGs were recorded after a perfusion period of 20 min. Note the reduction of activity when the drug concentration is increased in the artificial blood. For comparison a control recording is given.

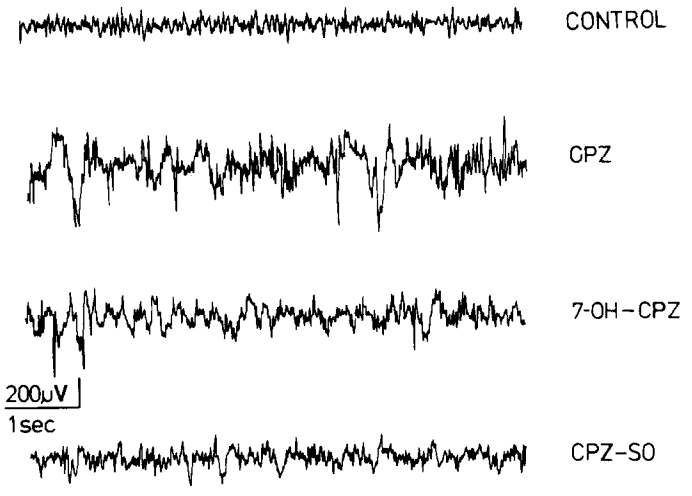

Fig. 2. EEG recordings from the isolated rat brain as influenced by CPZ, 7-OH-CPZ and CPZ-SO. Drug concentration in the artificial blood was 10 μM. Control EEG was obtained by omitting the drug. Since tracings were usually symmetrical and synchronous, the EEG of only one hemisphere is shown recorded after a perfusion period of 20 min.

control brain (Fig. 3). Perfusing CPZ the high frequency activity was significantly reduced and the theta and delta frequencies increased (Fig. 4). The changes in the frequency percentages produced by 7-OH-CPZ showed the same tendency but were not statistically significant. The percentage of alpha waves remained unchanged in all experiments. CPZ-SO did not produce significant shifts in the percentages of the EEG frequencies.

The amplitude increase indicated by amplitude histography was most pronounced after CPZ and was still statistically significant after 7-OH-CPZ (Fig. 5). CPZ-SO did not influence the EEG amplitude. The results of the statistical analysis of the EEG effects are summarized in Table 1.

HVA

The striatal HVA content of the isolated rat brain did not significantly differ from that in the rat brain *in vivo* (Table 2). The HVA level of the isolated brains increased markedly ($P < 0.01$) after perfusion with CPZ and, less pronounced, after 7-OH-CPZ. It was not significantly changed by CPZ-SO.

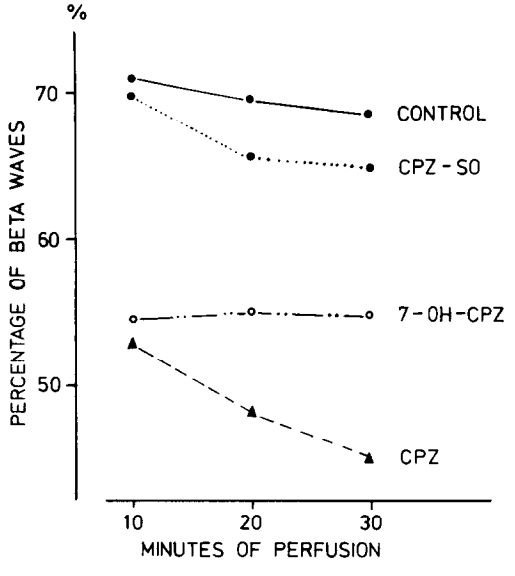

Fig. 3. Percentages of beta waves obtained from the interval histograms. Note the low initial level of beta activity during CPZ and 7-OH-CPZ treatment. No substantial change in controls. The points represent means of seven experiments. Statistical comparisons in Table 1.

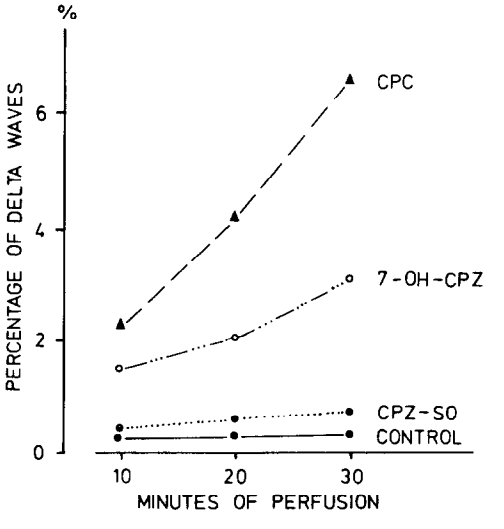

Fig. 4. Percentages of delta waves obtained from the interval histogram. Note the increase of delta activity produced by CPZ and 7-OH-CPZ. No substantial change during perfusion can be seen after administration of CPZ-SO and in controls. Each point represents the mean of seven experiments. Statistical comparison in Table 1.

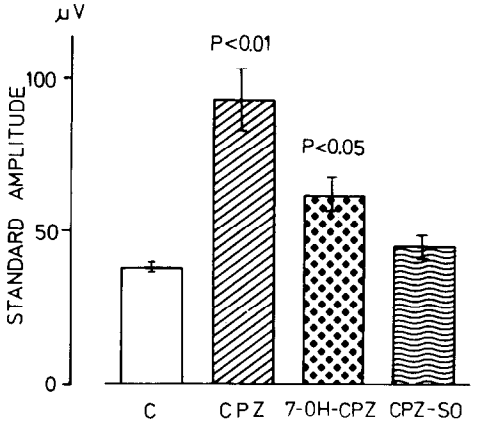

Fig. 5. Standard amplitude of the EEG as influenced by CPZ, 7-OH- CPZ and CPZ-SO. Drug concentration in the artificial blood was 10 μM. The standard amplitude was calculated from data of the amplitude histography after a perfusion period of 20 min. Means of seven perfusion experiments ± S.D. Statistical comparison in Table 1.

Correlation between the effects on the EEG and on the HVA level

Considering all experiments performed in this study, strong correlations were found between EEG data and the striatal HVA level in the isolated rat brain. With an increase in the HVA content there was a corresponding increase in the EEG amplitude and the percentages of the slow waves (theta and delta) and a decrease in the percentage of the beta waves. These correlations were highly significant (Table 3).

TLC

At the end of a perfusion experiment the phenothiazines were extracted from the simplified blood and were analyzed by TLC. Besides the agents applied, only traces of the corresponding sulfoxides could be detected.

DISCUSSION

As indicated by TLC the phenothiazines used are hardly metabolized in the isolated rat brain. Thus, we can start from the fact that the central effects analysed were actually produced by the agents applied and not by metabolites formed during perfusion.

Table 2. HVA levels in the striatum of the isolated perfused rat brain as influenced by CPZ, 7-OH-CPZ and CPZ-SO

Drug	HVA (μg/g wet tissue)
Control *in vivo*	0.336 ± 0.064
Control perfused	0.385 ± 0.062
CPZ	0.581 ± 0.100†
7-OH-CPZ	0.439 ± 0.154*
CPZ-SO	0.425 ± 0.061

The drugs were added to the artificial blood to give a final concentration of 10 μM. Perfusion time 30 min. Controls *in vivo* were brains of untreated rats killed by decapitation. Perfused controls were obtained by adding no drug to the artificial blood. The values are given as means of seven experiments ± S.D Significance of difference from the perfused control * $P < 0.05$, † $P < 0.001$.

Table 3. Correlation coefficients between EEG data and HVA levels in the striatum of the isolated rat brain

EEG parameter	Correlation coefficient
Standard amplitude	+ 0.757†
Beta %	− 0.531*
Alpha %	− 0.025
Theta %	+ 0.552*
Delta %	+ 0.583*

The EEG was recorded after a perfusion period of 20 min, HVA was determined at the end of perfusion (30 min perfusion period). The data of all perfusion experiments (controls and drug perfusions) were used to calculate the values which are presented as Spearman rank correlation coefficients. * $P < 0.01$, † $P < 0.001$

Although 8-OH-CPZ is able to cross the blood-brain barrier [14,15], only moderate EEG changes were found when the isolated brain was perfused with this metabolite. Neither 3,7-diOH-CPZ nor CPZ-SO significantly altered the control EEG. On the other hand, clear EEG effects were caused by 7-OH-CPZ and CPZ. The EEG profiles of these agents showed close similarities but with more marked changes after CPZ. These quantitatively different EEG effects were in accordance with the results of the HVA determination. The striatal HVA level in the isolated brain was most clearly increased after CPZ. The increase was less pronounced after

Table 1. Multiple bilateral comparisons of the EEG data

Comparison	Standard amplitude	frequency band beta	alpha	theta	delta
Control vs CPZ	↑ ↑	↓ ↓	n.s.	↑ ↑	↑ ↑
Control vs 7-OH-CPZ	↑	n.s.	n.s.	(↑)	n.s.
Control vs CPZ-SO	n.s.	n.s.	n.s.	n.s.	n.s.

Statistical significance was tested by the Nemenyi method [13]. ↑ ↑ $P < 0.01$, ↑ $P < 0.05$, (↑) $P < 0.1$, n.s. = not significant. Direction of arrows gives direction of change. The differences were calculated on the values of the second recording periods (20 min of perfusion).

7-OH-CPZ and was no longer significant after CPZ-SO. These results obtained from the isolated perfused rat brain are in accordance with the findings of several authors who demonstrated in various pharmacological and behavioral tests that 7-OH-CPZ possesses central activity similar to its parent compound CPZ [16–20]. 7-OH-CPZ seems to be the most active one out of the hydroxylated metabolites. The oxidation of the sulfur atom to form CPZ-SO obviously abolishes central activity.

Hornykiewicz [21] has reviewed evidence indicating that dopamine has an EEG activating action. In later reports the relationship between the dopamine system and the EEG has also been pointed out [22, 23]. If dopamine is actually important for maintaining arousals, it seems likely that a blockade of the dopamine receptors would result in EEG depression or synchronization. The slow wave patterns and the increase of the striatal HVA level caused by CPZ and 7-OH-CPZ in the isolated rat brain tend to support this idea.

As established by strong correlations calculated statistically from the data presented (Table 3), the acceleration of dopamine turnover in the striatum indicated by an increase of the HVA level is obviously accompanied by an increase in the EEG amplitude, the percentage of theta and delta waves as well as by a decrease in the percentage of beta waves.

In conclusion, it seems that 7-OH-CPZ is the most active hydroxylated metabolite of CPZ but is less active than its parent compound. Striatal dopaminergic neurons may be involved in the EEG effects caused by phenothiazines.

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REFERENCES

1. G. Sakalis, S. H. Curry, G. P. Mould and M. H. Lader, *Clin. Pharmac. Ther.* **13**, 931 (1972).
2. I. Creese, A. A. Manian, T. D. Prosser and S. H. Snyder, *Eur. J. Pharmac.* **47**, 291 (1978).
3. J. Krieglstein, H. Rieger and H. Schütz, *Eur. J. Pharmac.* **56**, 363 (1979).
4. R. Andjus, K. Suhara and H. A. Sloviter, *J. appl. Physiol.* **22**, 1033 (1967).
5. G. Krieglstein, J. Krieglstein and W. Urban, *J. Neurochem.* **19**, 885 (1972).
6. G. Krieglstein, J. Krieglstein and R. Stock, *Naunyn-Schmiedeberg's Archs. Pharmac.* **275**, 124 (1972).
7. W. Fleck, J. Krieglstein and W. Urban, *Arzneimittel-Forsch.* **22**, 1225 (1972).
8. J. Grüner, J. Krieglstein and H. Rieger, *Naunyn-Schmiedeberg's Archs. Pharmac.* **277**, 333 (1973).
9. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
10. N. E. Anden, B. E. Roos and B. Werdinius, *Life Sci.* **2**, 448 (1963).
11. M. G. Saunders, *Electroenceph. clin. Neurophysiol.* **15**, 761 (1963).
12. B. Saltzberg and N. R. Burch, *IRE Trans. med. Electron.* **8**, 24 (1957).
13. L. Sachs, *Angewandte Statistik,* Vol 4, Springer, Berlin (1974).
14. A. A. Manian, D. H. Efron and S. R. Harris, *Life Sci.* **10**, 679 (1971).
15. R. P. Maickel, N. M. Fedynskyj, W. Z. Potter and A. A. Manian, *Toxicol. appl. Pharmac.* **28**, 8 (1974).
16. A. A. Manian, D. H. Efron and M. E. Goldberg, *Life Sci.* **4**, 2425 (1965).
17. S. Lal and T. L. Sourkes, *Eur. J. Pharmac.* **17**, 283 (1972).
18. H. Barry III, M. L. Steenberg, A. A. Manian and J. P. Buckley, *Psychopharmacologia (Berl.)* **34**, 351 (1974).
19. R. L. Bronaugh and M. Goldstein, *Psychopharmac. Commun.* **1**, 201 (1975).
20. H. Y. Meltzer, V. S. Fang, M. Simonovich and S. M. Paul, *Eur. J. Pharmac.* **41**, 431 (1977).
21. O. Hornykiewicz, *Pharmac. Rev.* **18**, 925 (1966).
22. V. Florio and V. G. Longo, *Neuropharmacology* **10**, 45 (1971).
23. M. R. Džoljić, I. L. Bonta, M. Godschalk, A. Lagendijk and S. Stefanko, *Neuropharmacology* **14**, 591 (1975).