The biochemical origins of the enzymatic activity increase itself, whether in our particular case, or in those mentioned in the introductary paragraph, remains problematic: several authors, already cited, advance the hypothesis that such increases in activity result from an increase in membrane permeability (presumably due to an influence of corticosteroids[16,17]), without however producing supporting evidence. Likewise, the light decrease in hematocrit observed during the experiments does not explain the pattern of plasma CPK activity, and particularly the existence of a peak of activity.

The appearance of a maximum between the 21st and the 24th experimental h might correspond to an infradian rhythm of stress-susceptibility[18], inducing a similar rhythm of plasma CPK activity; however, as this phenomenon did not recur during the 81 experimental h, such a hypothesis can scarcely be retained. It seems more logical that such an activity pattern represents an adaptation by the animals to experimental conditions, causing, after an increase, a decrease in the release of the enzyme, since a phenomenon as rapid cannot be explained by the modulation of the mechanism of synthesis and utilization of the CPK at organism level.

Therefore, although these results are pertinent only for rabbits, the interference of numerous factors with serum or plasma CPK activity (and particularly the stress accompanying blood sampling in rabbits) would seem to indicate that a certain care is necessary in the interpretation of results in physiology and experimental pathology[19], or of certain clinical cases[20,21] concerned with this enzyme[22,23].

[16] F. SCHAPIRA, C. r. Soc. Biol., Paris *148*, 1997 (1954).
[17] F. SCHAPIRA, personal communication (1975).
[18] F. HALBERG and A. REINBERG, J. Physiol., Paris *59*, 117 (1967).
[19] P. D. ALTLAND, B. HIGHMAN and R. G. SELLNER, Cryobiology *11*, 296 (1974).
[20] J. ROGET, A. BEAUDOING, J. JOBERT and M. BOST, Pédiatrie *24*, *163* (1969).
[21] K. S. PHORNPHUTKUL, S. ANURAS, R. S. KOFF, L. B. SEEF, D. L. MAHLER and M. J. ZIMMERMAN, Clin. Chem. *20*, 340 (1974).
[22] R. S. GALEN and S. R. GAMBINO, Clin. Chem. *21*, 272 (1975).
[23] J. C. TURPIN, Ann. Pediat. *22*, 107 (1975).

Effect of 6-Hydroxydopamine Pretreatment on Spontaneous Convulsions Induced by Barbital Withdrawal[1]

W. W. MORGAN[2]

University of Texas, Health Sciences Center at San Antonio, Department of Anatomy, 7703 Floyd Curl Drive, San Antonio (Texas 78284, USA), 16 October 1975.

Summary. Following withdrawal from chronic barbital administration, 6-hydroxydopamine pretreated rats show a greater number and an earlier onset of spontaneous convulsive seizures than do rats pretreated with the saline-ascorbic acid vehicle.

Abrupt withdrawal following the long term intake of barbiturates results in an abstinence syndrome characterized by the occurrence of spontaneous convulsive seizures in man (ISBELL et al.[3]) and in rats (ESSIG[4]). The biochemical mechanism underlying the appearance of these spontaneous convulsions has been only minimally investigated. Acute treatment with anesthetic dosages of of pentobarbital has been shown to decrease the turnover of noradrenaline and dopamine (CORRODI, FUXE and HÖKFELT[5], PERSSON and WALDECK[6]). Changes in the activity of noradrenaline and/or dopamine containing neurons following the long term administration of barbiturates could be related to some of the symptoms of the abstinence syndrome, i.e. spontaneous convulsions. Thus it was of interest to determine the effect that the chemical lesioning of brain noradrenaline and to a lesser extent brain dopamine nerve ending with 6-hydroxydopamine would have on the onset and incidence of spontaneous convulsions in rats withdrawn following chronic barbital treatment.

Materials and methods. A small polyethylene cannula (PE10) was implanted into each lateral ventricle of adult male Sprague-Dawley rats. The cannulae were secured in place with dental cement and 2 stainless steel screws implanted into the skull. Each cannula was kept patent by the use of a stainless steel stylet of the exact length to extend just to the tip of the cannula. 40 rats subsequently referred to as 6-hydroxydopamine pretreated were anesthetized with ether and given 100 µg of 6-hydroxydopamine hydrobromide (6-HODA) (Sigma) in 20 µl of saline (1 mg ascorbic acid per ml) in each lateral ventricle. 40 rats subsequently referred to as saline treated were also anesthetized and received only the saline vehicle. The treatment was repeated on alternate days for a total of 3 injections. After the 3rd injection, the stylets were removed, the cannulae were clipped off next to the skull and then plugged with dental cement. The animals were housed 4 per cage and exposed to a 14:10 light dark lighting regime (lights on 06.00–20.00 h). 1 week later 32 of the 6-HODA pretreated and 23 of the saline pretreated control rats were started on a dosage regimen of increasing concentrations of barbital in the drinking water. The lower sample sizes reported in the results section reflect the loss of animals during the 6 weeks of the barbital regimen. The initial concentration of barbital (not sodium salt) was 1 mg/ml which was given for 3 days. The bitter taste of barbital was disguised with saccharine (20 mg/l). The control or non-barbital treated rats were given the same concentration of saccharine in their

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[3] H. ISBELL, S. ALTSCHULE, C. H. KORNETSKY, A. J. EISENMAN, H. G. FLANNARY and H. F. FRASER, Arch. Neurol. Psychiat. *64*, 1 (1950).
[4] C. F. ESSIG, Neuropharmacology *5*, 103 (1966).
[5] H. CORRODI, K. FUXE and T. HÖKFELT, J. Pharm. Pharmac. *18*, 556 (1966).
[6] T. PERSSON and B. WALDECK, J. Pharm. Pharmac. *23*, 277 (1971).

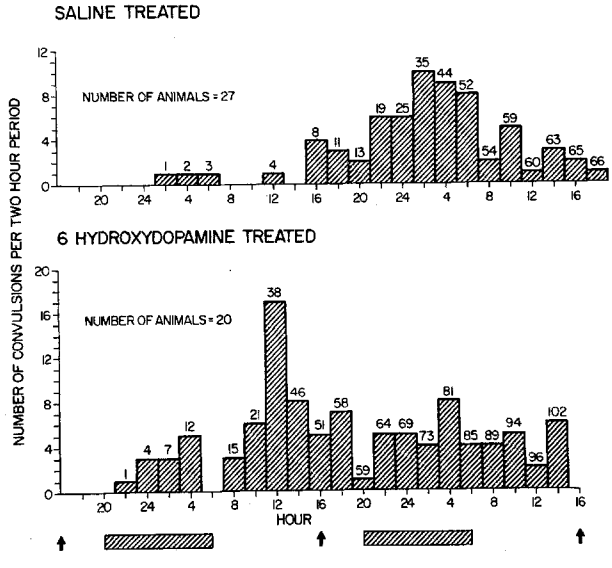
Comparison of effects of 6-hydroxydopamine or saline pretreatment on the content of noradrenaline and dopamine in the telencephalon and on the body weights of barbital dependent rats

Treatment	Body Weight (g)	Noradrenaline content (μg/g telencephalon)	Dopamine content (μg/g telencephalon)
A) 6-HODA + barbital	322 ± 9 (20)[8]	0.02 ± 0.01 (4)[9]	0.28 ± 0.06 (9)
B) Saline + barbital	401 ± 4 (27)	0.24 ± 0.02 (10)	1.00 ± 0.03 (10)
C) 6-HODA	350 ± 18 (5)	0.02 ± 0.01 (4)[9]	0.29 ± 0.03 (4)
D) Control	363 ± 12 (8)	0.28 ± 0.02 (7)	1.00 ± 0.02 (7)
	A < B $p < 0.001$[10]; A < D $p < 0.005$ B > C $p < 0.005$; B > D $p < 0.005$	A < B and D $p < 0.001$; C < B and D;	A < B and D $p <$ 0.001; C < B and D.

The number in parentheses indicates the sample size.

drinking water. The dosage of barbital was then increased at 0.5 mg/ml increments with the number of days per dosage increasing by 1 with each increment in barbital concentration. The saccharine concentration was also increased by 10 mg/ml with each increment in barbital concentration. The highest concentration of barbital administered was 4 mg/ml for 8 days. The barbital treated rats were abruptly withdrawn at 16.00 h and with the control rats were observed continuously for 48 h. Convulsive seizures were classified as a wild run or as clonic or tonic. At the end of 48 h, one half of the surviving barbital treated rats and all of the non-barbital treated rats were sacrificed, and the telencephalon was dissected for subsequent analysis of dopamine and noradrenaline content (SHELLENBERGER and GORDON[7]).

Results. The accumulative number, total number and the time of occurrence of spontaneous convulsive seizures in saline pretreated versus 6-HODA pretreated rats following barbital withdrawal are shown in the Figure. Exactly half of the 102 convulsive seizures observed in the 6-HODA pretreated rats during the first 48 h following barbital withdrawal were observed during the first 24 h of this period. During the first 24 h following barbital withdrawal, 19 of the 20 6-HODA pretreated rats convulsed at least once. Of the 51 convulsive seizures observed in the 6-HODA pretreated group during the first 24 h, 28 were wild runs, 12 were clonic and 11 were tonic seizures. In the same group during the second 24 h 10 wild runs, 26 clonic and 15 tonic seizures were observed. A total of 102 convulsive seizures were observed in 20 6-HODA pretreated animals for a mean of 5.1 convulsions per animal.



Comparison of the effects of 6-hydroxydopamine or saline pretreatment on the incidence and the time of onset of spontaneous convulsions following withdrawal of barbital dependent rats. The abscissa indicates the clock hours following barbital withdrawal. The diagonal line bar below the abscissa indicates the dark phase of the 14:10 LD lighting regime while the black vertical arrows delineate the 24 h periods following withdrawal. The ordinate indicates the number of convulsions observed in 6-hydroxydopamine and saline pretreated rats, respectively. The bar graph shows the number of convulsions per 2 h period while the number over each bar indicates the cumulative number of convulsions observed from the time of withdrawal.

On the other hand, only 8 convulsive seizures were observed in the saline pretreated rats during the first 24 h following with-drawal. Of the 8 convulsions observed 1 was a wild run, 6 were clonic and 1 was a tonic seizure. During the second 24 h following barbital withdrawal, 58 convulsions were observed in the saline pretreated animals. 5 of these convulsions were wild runs, 50 were clonic and 3 were tonic seizures. A total of 66 convulsive seizures were observed in 26 of 27 saline treated rats for a mean of 2.5 convulsions per animal.

48 h of barbital withdrawal alone had no effect on noradrenaline or dopamine content in the telencephalon or the brainstem of saline treated or 6-HODA pretreated rats (Table). On the other hand, pretreatment with 6-HODA significantly decreased noradrenaline ($p <$ 0.001) in the telencephalon.

The saline pretreated rats which were given barbital had heavier body weights than those animals not given barbital (Table). On the other hand, rats pretreated with 6-HODA and then given barbital had smaller body weights than any other group (Table). The body weights of rats pretreated with 6-HODA but not given barbital were not significantly different from those of control rats.

Discussion. The most striking observation of this study was the markedly earlier onset of spontaneous convulsive seizures in those barbital dependent rats whose noradrenaline and dopamine levels were markedly decreased by pretreatment with 6-HODA. The barbital dependent rats which had been pretreated with 6-HODA also had a greater number of convulsive seizures during the first 48 h

[7] M. K. SHELLENBERGER and J. H. GORDON, Analyt. Biochem. *39*, 356 (1971).

[8] The number in parentheses indicates the sample size.

[9] The fluorescence of telencephalon noradrenaline in number of 6-hydroxy-dopamine treated animals was not twice the blank and thus were not included in the calculation.

[10] R. R. SOKAL and F. J. ROHLF, *Biometry* (W. H. Freeman and Company, San Francisco 1969), p. 208.

following withdrawal. The type of convulsive seizures observed also seemed to be influenced by 6-HODA pretreatment since nearly equal numbers of wild runs, clonic and tonic seizures were observed in these animals while clonic seizures were by far the most common type observed in saline pretreated rats.

Earlier studies have also demonstrated close correlations between convulsive seizure susceptibility and low brain noradrenaline. SCHLESINGER and BOGGAN[11] showed a correlation between age dependent audiogenic seizure susceptibility and brain noradrenaline content. REITER and MORGAN[12] demonstrated a close correlation between the convulsion susceptibility of parathyroidectomized rats following subsequent pinealectomy and a decrease in brain noradrenaline. ARNOLD, RACINE and WISE[13] demonstrated an increase in sensitivity to electrically induced convulsive seizures following noradrenaline depletion with 6-HODA.

Those rats which were first pretreated with 6-HODA and then treated chronically with barbital had significantly lower body weights than either control rats or rats pretreated with saline and then given barbital. Previous investigators have shown that the intraventricular administration of 6-HODA results in a decrease in food consumption and a lower body weight[14]. In this study rats which received only 6-HODA pretreatment did not have significantly lower body weights than controls, but this may be related to the very low sample size of this particular group. In this study chronic barbital treatment alone resulted in significantly higher body weights. Further studies will be required to determine if this is a consistent finding and if it is secondary to a stimulation of food consumption. The present data show that the depletion of brain noradrenaline and dopamine causes a markedly earlier onset and a somewhat greater incidence of spontaneous seizures following the withdrawal of barbital dependent rats. From the present data, it is not possible to determine which of these 2 catecholamines is more important in producing this effect. On the other hand, it remains to be shown that a decrease in the activity of either a noradrenaline or a dopamine pathway in the brain is responsible for the spontaneous convulsive seizures observed following barbital withdrawal.

[11] K. SCHLESINGER, W. BOGGAN and D. X. FREEDMAN, Life Sci. *4*, 2345 (1965).
[12] R. J. REITER and W. W. MORGAN, Physiol. Behav. *9*, 203 (1972).
[13] P. S. ARNOLD, R. J. RACINE and R. A. WISE, Expl Neurol. *40*, 457 (1973).
[14] G. R. BREESE, R. D. SMITH, B. R. COOPER and L. D. GRANT, Pharmac. Biochem. Behav. *1*, 319 (1973).

Inhibition of Ovulation in Rats by Antagonists to Serotonin and by a New Tricyclic Compound

M. MARKÓ and E. FLÜCKIGER

Biological and Medical Research Division, Department of Pharmacology Sandoz Ltd., CH–4002 Basel (Switzerland), 5 November 1975.

Summary. The ovulation inhibiting activity in adult rats of the 5HT-antagonists cyproheptadine, mianserin and methysergide is shown. Furthermore the activity of a newly synthetized Cycloheptathiophenederivative, compound 26–921, which inhibits LH-secretion and consequently ovulation, is described.

In recent years, a considerable amount of information has been obtained concerning the neuroendocrine control of anterior pituitary function. It is assumed that changes in amine metabolism in discrete parts of the brain influence the secretion of gonadotropines and consequently the process of ovulation[1]. The effect of pharmacologically-induced changes in adrenergic transmission have strengthened this assumption[2]. Relatively little work has been done towards analyzing the role of serotonin (5HT) in the control of ovulation. BROWN[3] reported that serotonin antagonists such as LSD and methysergide[4,5] inhibit PMS-induced ovulation in immature mice. As we have observed profound differences between the pharmacological responsiveness of PMS-induced ovulation as compared to spontaneous ovulation[6], it seemed necessary to investigate whether some well known 5HT-antagonists are active in spontaneously ovulating adult rats also.

Here we wish to report on the ovulation inhibiting activity of these 5HT-antagonists: besides methysergide, an ergot derivative, compounds of different chemical classes were included. Further we describe the activity of a newly synthetized cycloheptathiophene-derivative, compound 26–921[7].

Material and methods. Adult female rats of the Ivanovas Wistar strain (200–250 g) were used in our experiments. The conditions of experimentation were the same as described recently[6]. The animals were injected s.c. in prooestrus at noon with the following compounds: Compound 26–921, 9,10-dihydro-10-methyl-4-(1-methyl-4-piperidyliden)-4-H-*benzo* (4,5) *cyclohepta* (1,2-b) *thiophen*-hydrogenmalate, dissolved in saline; methysergide-hydrogenmalate, dissolved in alcohol and tartaric acid; cyproheptadine-hydrochloride (MS&D), and mianserin-hydrochloride (Organon), both dissolved in saline. In oestrus at 09.00 h the rats were sacrificed and ova were counted in both Fallopian tubes with the aid of a dissecting microscope. Only when no eggs were found, was ovulation considered to be inhibited. Mean number of eggs per ovulating rat in each treatment group was calculated. From the proportion of rats ovulating in any treatment group the 50% inhibitory dose (ED_{50}) was calculated using the method of LITCHFIELD and WILCOXON[8]. In another series of experiments, compound 26–921 0.3 mg/kg s.c. was injected in prooestrus-rats at noon, controls receiving 0.9% saline solution. The animals were decapitated either at 18.00 h of the same day, or at

[1] C. A. WILSON, in *Advances in Drug Research* (Ed. N. J. HARPER and A. B. SIMMONDS; Academic Press, London 1974), p. 119.
[2] F. PIVA, N. STERESCU, M. ZANISI and L. MARTINI, Bull. Wld. Hlth. Org. *41*, 275 (1969).
[3] P. S. BROWN, J. Endocr. *37*, 327 (1967).
[4] W. DOEPFNER and A. CERLETTI, Int. Arch. Allergy *12*, 89 (1958).
[5] E. FLÜCKIGER and R. SALZMANN, Experientia *17*, 131 (1961).
[6] M. MARKÓ and E. FLÜCKIGER, Experientia *30*, 1174 (1974).
[7] J. M. BASTIAN and M. MARKÓ, Experientia *32*, 413 (1976).
[8] J. T. LITCHFIELD and F. WILCOXON, J. Pharm. *96*, 99 (1949).