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Influence of Loxapine on the Sleep-Wakefulness Cycle of the Rat[1]

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SCHMIDEK, W. R., C. TIMO-IARIA, M. SCHMIDEK, M. KRAKOWIAK, M. R. ALVES AND E. E. DELMUTTI. *Influence of Loxapine on the sleep–wakefulness cycle of the rat.* PHARMAC. BIOCHEM. BEHAV. **2**(6) 747–751, 1974. – The sleep–wakefulness cycle was studied in 18 Wistar albino rats under the influence of Loxapine, a neuroleptic derived from oxazepine succinate. One single dose of the drug (0.4–1.6 mg/kg) had a marked effect in depressing the paradoxical sleep (PS), maximal within 30 min and lasting about 24 hr. Frequency and mean duration of PS episodes were differentially affected. Synchronized sleep was only slightly affected by Loxapine whereas no significant changes of wakefulness amount were detected. Chronic administration of the drug induced similar changes which disappeared in about 5 days.

Pharmacology of sleep Drugs and sleep Loxapine Paradoxical sleep

PHARMACOLOGICAL influences on the sleep–wakefulness cycle have been widely studied in recent years. The identification of humoral factors involved in the neural mechanisms underlying sleep [9] and the demonstration that some drugs (especially those which are known to affect the nervous system, mainly exhibiting psychotropic properties) specifically change particular phases and states of the cycle [7, 9, 16, 21] emphasize the potentiality of the pharmacological approach for a better understanding of the neurophysiology of sleep.

In the present study the neuroleptic 2-chloro-11 (4-methyl-1-piperazinyl)dibenz (b,f) (1,4) oxazepine succinate (Loxapine, Cyanamid International), was assayed as to its possible influences on the sleep–wakefulness cycle in the rat. This drug facilitates polysynaptic reflex potentials at the spinal level and seems to have a facilitatory effect on subcortical sensory reception areas [14]. Epileptogenic activity has also been reported to occur in cats when high doses were used whereas with low doses a decrease of alertness, exploratory behavior and motor responses to sensory stimulation, and drowsiness were reported [5]. Besides, Loxapine has been proved effective as an antipsychotic [1]. The actions of this drug seems, therefore, to be exerted on several neural systems; thus, interference with sleep could be considered possible. In fact, our experiments showed that Loxapine strongly and reversibly influences sleep in the rat, affecting mainly the desynchronized state.

METHOD

Animals

The experiments were performed on 18 adult, male Wistar albino rats, prepared for chronic recording of the electrocorticogram from frontal areas, electromyogram of dorsal neck muscles, and eye movements, according to the technique described elsewhere [15,23]. All animals were previously adapted to 24° C in a thermic chamber [20].

Procedure

Recordings. For recordings the rats were kept in a small Faraday cage (0.20 × 0.10 × 0.09 m), inside of which temperature was maintained constant at 28° C. Electrocorticogram, electromyogram and eye movements were continuously recorded with a Beckman polygraph during 90 min. The period of recording was selected between 10 a.m. and 5 p.m., beginning 4 days after implantation of the electrodes. For analytical purposes the three states of the sleep–wakefulness cycle were considered: wakefulness, synchronized sleep, and desynchronized or paradoxical sleep. The following parameters were measured: (1) total

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amount of each state during the recording period; (2) frequency of episodes; (3) mean duration of the episodes. The values of these variables were expressed as the ratio after drug value/control value.

Experimental schedule. (1) Acute administration of the drug. Three lots of 4 rats received intraperitoneally one of the following doses of Loxapine: 0.4, 0.8 or 1.6 mg/kg. Recordings started 0.5, 5, 24 and 72 hr after injection. The same animals used for experimental tests were used as controls; they received an equivalent volume of saline by the same route, which was followed by recordings after the same time intervals as above. The control schedule preceeded or followed, at random, the after drug schedule. Each rat was subjected only to 1 dose of Loxapine, with all recordings being performed within 15 days. (2) Chronic administration. Six rats received for 12 days a daily dose of 1.6 mg/kg of Loxapine; out of these, 4 were selected at random in each day and subjected to sleep recordings, 5 hr after the administration of the drug. The same rats were used as controls by being given an equivalent volume of saline, followed by recordings after the same time interval as above. Two or 3 control recordings were performed for each rat, preceeding the after drug experiment. From these a mean value was taken for comparison with all the after drug values. For these experiments a polyethylene tube (PE 50) was chronically implanted in the peritoneal cavity and made to emerge through the back skin of the neck, to allow repeated injections.

Statistical analysis. Data obtained from all the experiments were statistically treated. Rejection level of null hypothesis was adopted as $\alpha = 0.05$. All variables were assumed homossedastic and normally distributed. Analysis of variance was employed to check the differences between the means. Individual mean values were compared to constant 1.00 value by t-test [22].

RESULTS

Wakefulness

Although the means of the amount of wakefulness, when the rats received Loxapine, showed some variations (Fig. 1) these were not statistically significant, $F(3,34) = 0.62$ and $F(2,34) = 1.33$, for time and dose effects, respectively. In spite of this, behaviorally the animals clearly exhibited qualitative signs of being affected by the drug; in less than half an hour after the injection was performed the rats became quiet and showed little or no exploring of the environment or grooming; when present, both behaviors were performed slowly. For the most part of the time they adopted a posture we termed stagnant because they usually lay quietly on the floor of the cage, with semi-closed eyes and reacting considerably less than before to environmental stimulation. Such behavior, characteristically present during the early hours after administration of the drug (especially when the highest dose – 1.6 mg/kg – was given), disappeared within the first 24 hr.

Synchronized Sleep (SS)

As shown in Fig. 1 the effects of Loxapine on synchronized sleep were mild but statistically significant $F(3,24) = 6.00$, $p<0.01$, and $F(2,34) = 3.67$, $p<0.05$, for time and dose effects, respectively. However, though the 3 groups showed slight initial increases and subsequent reductions, none of the individual shifts were significant.

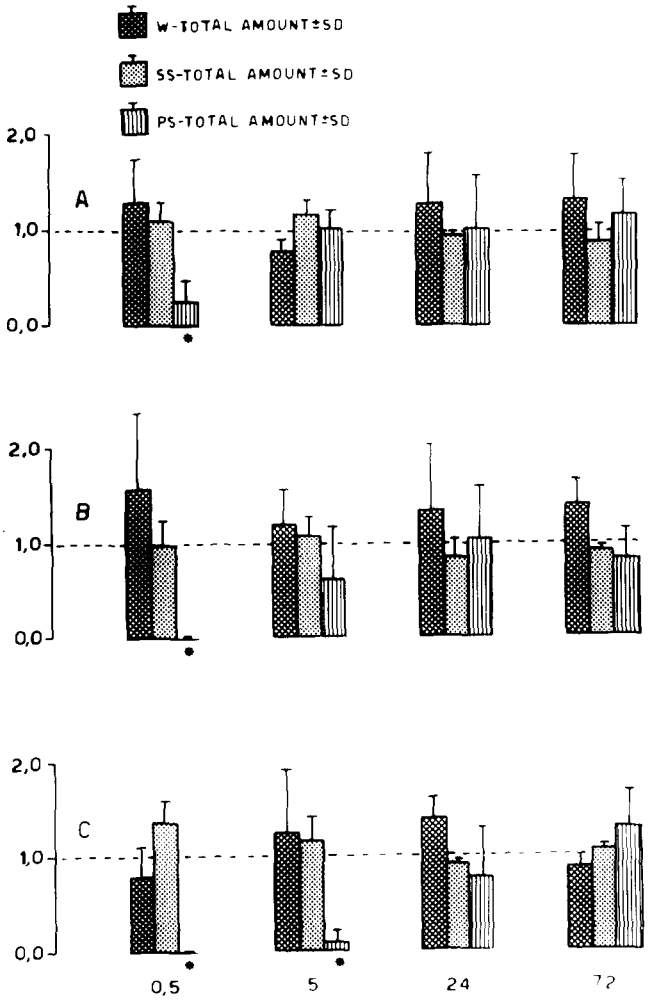

FIG. 1. Acute administration of Loxapine. Total amounts of wakefulness (W), synchronized sleep (SS), and paradoxical sleep (PS). Ordinates: mean relative values (after drug value/control value) of each parameter ± SD (n = 4). Abscissae: time (in hours) after one single injection of the drug (or of saline for controls). A, B, and C: effects of 0.4, 0.8, or 1.6 mg/kg of Loxapine, respectively. *: $p<0.05$.

Paradoxical Sleep (PS)

Both Figs. 1 and 2 show quite clearly that desynchronized sleep was deeply affected by Loxapine. Following a single injection of the drug the total amount of PS decreased intensely, $F(3,24) = 14.92$, $p<0.001$ and $F(2,24) = 2.74$, pNS for time and dose effects respectively. The effect was prompt, since it attained the highest degree within the early 0.5 hr after administration. Individual contrasts for this first half hour in the three groups (0.4, 0.8, and 1.6 mg/kg) were, respectively: $t = 6.71$, $df = 3$, $p<0.01$; $t>100$, $df = 3$, $p<0.001$; $t>100$, $df = 3$, $p<0.001$. Partial or complete recovery occurred within 5 to 24 hr and the higher the dose the more prolonged was the decrease of PS. Individual contrasts for the 5 hour values were only significant for the 1.6 mg/kg group ($t = 14.10$, $df = 3$, $p<0.001$). For the other values (24 and 72 hr) t was not significant.

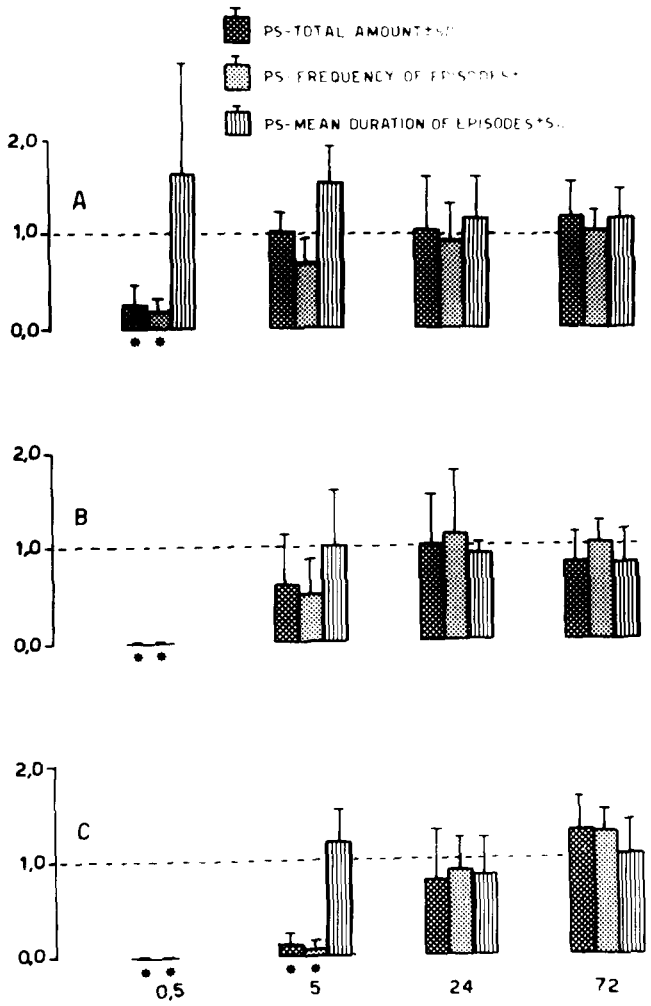

FIG. 2. Acute administration of Loxapine. Paradoxical sleep (PS): total amount, frequency of episodes, and mean duration of episodes. Ordinates: mean relative values (after drug value/control value) or each parameter ± SD (n = 4). Abscissae: time (in hours) after one single injection of the drug (or of saline for controls). A, B, and C: effects of 0.4, 0.8, or 1.6 mg/kg of Loxapine, respectively. *: $p<0.05$.

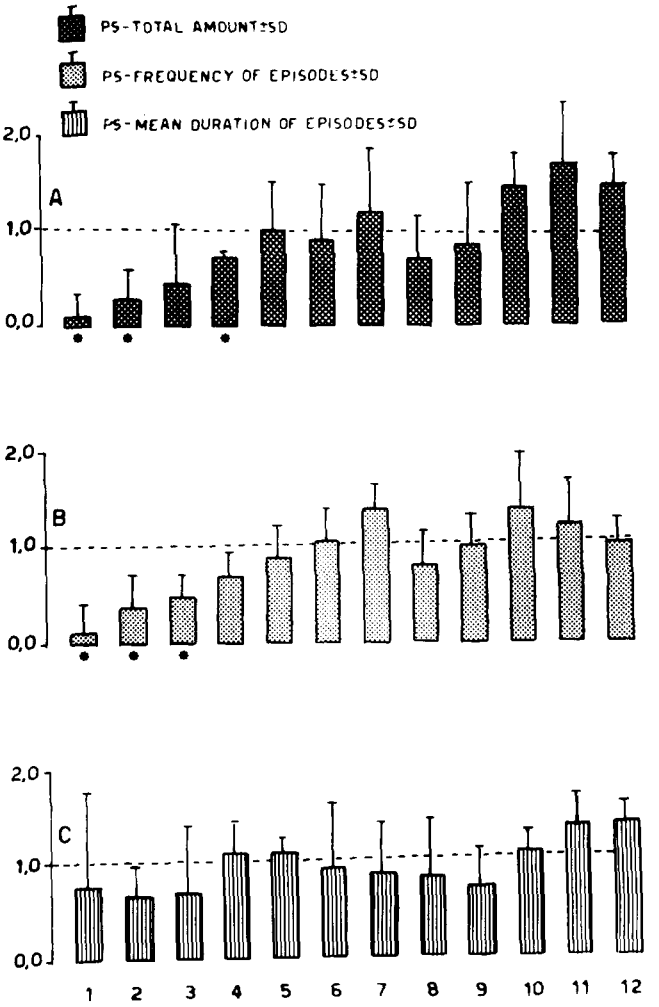

FIG. 3. Chronic administration of Loxapine. Paradoxical sleep (PS): total amount, frequency of episodes, and mean duration of episodes. Ordinates: mean relative values (after drug value/control value) of each parameter ± SD (n = 4). Abscissae: consecutive days of administration of 1.6 mg/kg/day of Loxapine. *: $p<0.05$.

Note, however, the nonsignificance of the dose effect, as described above.

When frequency and mean duration of PS episodes were separately plotted it became evident that these two parameters were differentially affected by the drug. As shown in Fig. 2 the former decreased steeply, maximally within 30 min, and then gradually returned to normal; this time-dependent variation is statistically highly significant, $F(3,34) = 29.70$, $p<0.001$, and $F(2,34) = 1.25$, pNS for time and dose effects. Individual contrasts showed significant differences, (a) for the first half hour values in the 3 groups (0.4, 0.8, and 1.6 mg/kg), respectively $t = 11.74$, $df = 3$, $p<0.01$; $t>100$, $df = 3$, $p<0.001$; $t>100$, $df = 3$, $p<0.001$; and (b) for the five hour values in the 1.6 mg/kg group: $t = 18.40$, $df = 3$, $p<0.001$. Mean duration, on the other hand, did not show such an effect, since there were no statistically meaningful differences. In the first group (0.4 mg/kg) there was even an increase of the means of this parameter during the first 0.5 through 5 hr (though not significant). Note that mean duration for the first 0.5 hr in the other two groups (0.8 and 1.6 mg/kg) could not be determined for obvious reasons, since PS episodes were supressed.

Chronic administration of Loxapine also affected desynchronized sleep (Fig. 3). The total amount of PS, when the drug was injected chronically (1.6 mg/kg/day) and recording was performed 5 hr after injection, did not remain constant, $F(11,33) = 4.22$, $p<0.01$. There was a strong inhibition of PS in the first day, which gradually decreased to attain normal values by the fourth or fifth day, in spite of administration of Loxapine being continued, and then an increase occurred, reaching supranormal levels. Individual contrasts for the first 4 days were, respectively, $t = 7.62$, $df = 3$, $p<0.01$; $t = 4.89$, $df = 3$, $p<0.02$; $t = 1.65$, $df = 3$, pNS; $t = 17.64$, $df = 3$, $p<0.001$;

all other contrasts were not significant. The effects of the drug on frequency and mean duration of PS episodes (Fig. 3) again showed a differential influence. Frequency variations were, as in acute administration experiments, parallel to those of the relative amount, whereas differences between the values of mean duration were not statistically significant. Statistical data for PS frequency were $F(11,33) = 12.76$, $p<0.001$, with individual contrasts for the 3 first days of $t = 5.67$, $df = 3$, $p<0.02$; $t = 3.92$, $df = 3$, $p<0.05$; $t = 3.95$, $df = 3$, $p<0.05$; all other contrasts were not significant.

DISCUSSION

The results above described show that Loxapine intensely but reversibly interferes with the sleep–wakefulness cycle; it is the desynchronized sleep that is most affected by the drug, whose influence on synchronized sleep is very mild and on wakefulness is null.

The slight and inconstant, though significant, SS amount variations are difficult to interpret. Reduced and narrow numerical differences as well as low significance level, as compared to changes of PS amount, suggest a weak influence of Loxapine upon this state. The initial increase could perhaps be best explained as a consequence of intense PS inhibition, without concomitant interference with the wakefulness mechanisms.

By far and large the effects of Loxapine on desynchronized sleep were much more intense than on SS, both for acute and chronic administration of the drug. In the latter series of experiments, however, the depressing effect of Loxapine on PS decreased, allowing a return to normal levels within 4–5 days, which was followed by an overshoot in the subsequent days. The change in the effect of the drug could be accounted for either by habituation to the drug or by cumulative PS deprivation. The latter hypothesis explains better the overshoot of the PS amount during the last days (although these contrasts were not significant there appears a tendency for being so, which might turn into a full significance if the number of experiments were larger).

The sensitivity of desynchronized sleep to Loxapine is not a unique phenomenon since PS is clearly the most vulnerable of the two states of sleep. It is well known that barbiturates, when given in anesthetic doses never induce paradoxical sleep, though producing a state of unconsciousness that is electroencephalographically very similar to physiological sleep. A broad group of drugs influence sleep parameters [7,16], a large sub-group of which exerts a specific depressing effect on the paradoxical sleep. In this sub-group are included drugs with quite different structures, such as amphetamines [19] and its derivatives, barbiturates [15,17], ethyl alcohol [4], other hypnotics as meprobamate [3] and nitrazepan [18], diphenylhydantoin [2], imipramine and its derivatives [6], morphine [11], probenecid [13], monoamine oxydase inhibitors [10]. Our results indicate that Loxapine can be included in the subgroup of drugs that affect PS. Since a clear knowledge of the neural action sites and the mechanisms involved for most of these drugs is still lacking (and that obviously includes the presently studied Loxapine), there are few possibilities of inserting them all at the moment in a comprehensive discussion of sleep mechanisms. Exception could perhaps be made for those drugs which affect the brain biogenic amines metabolism [9]. Jouvet hypothetically relates the PS control to priming serotonergic and to triggering serotonergic, cholinergic and noradrenergic mechanisms, discussing the effects of some drugs on sleep (p-chlorophenylalanine, tryptophan, monoamine oxydase inhibitors, atropine, α-methyl-p-tyrosine, disulfiram, α-methyldihydroxyalanine) as to their possible interference with these mechanisms [9]. If the theory put forward by Jouvet is primarily correct one might suppose that most drugs affecting PS could be directly or indirectly interfering with biogenic amines in the centers which control sleep. Loxapine, however, apparently does not interact with biogenic amines in the central nervous system of rodents (Cyanamid Preclinical Brochure, Cyanamid International), and thus this reasoning cannot be generalized. A study of Loxapine distribution in the nervous tissue showed a greater affinity for diencephalic and mesencephalic structures than for cortex, cerebellum and cervical cord (Cyanamid Preclinical Brochure, Cyanamid International). The key structures involved in PS are apparently located in the rhombencephalon [8], which suggests that Loxapine may exert its action in subsystems related to the primary centers which control PS and these may be located in midbrain and diencephaoln.

Attention should be drawn to the dissimilar influence of Loxapine on frequency and mean duration of PS episodes. As described above, the former parameter had a steep, short latency and long lasting decrease, paralleling the variations of the PS amount. Mean duration, on the other hand, showed no such influence, neither under acute nor under chronic administration; in one of the acute administration groups (0.4 mg/kg) the effect was even opposite, with an initial, though not significant rise of this parameter. Such results fit well into the hypothesis [20] of partially independent control mechanisms for frequency and for duration of PS episodes. The partial independence of these parameters of desynchronized sleep is suggestive that they have to be coupled by some controlling system for the total amount of PS to be constant; thus, the constancy of the amount of this important state of sleep [12] (which is obviously a function of frequency and mean duration of the episodes) could be achieved by a selective control of any of these two mechanisms.

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