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EFFECTS OF OXOLINIC ACID ON THE SLEEP-WAKEFULNESS CYCLE OF THE RAT

L. D'ANGELO & J.M. MONTI

Department of Pharmacology and Therapeutics, School of Medicine, Clinics Hospital, Montevideo, Uruguay

1 A study was carried out in rats (prepared for chronic sleep recording) of the effects of oxolinic acid on the sleep-wakefulness cycle.

2 In addition, the actions of oxolinic acid on the sleep-wake cycle were assessed after pretreatment with drugs interfering with central catecholamine mechanisms or facilitating central γ-aminobutyric acid (GABA) activity.

3 Oxolinic acid (8–32 mg/kg) induced a significant and dose-related increase of waking EEG, while slow wave and REM sleep were decreased.

4 The effects of oxolinic acid on waking, slow wave and REM sleep were antagonized by α-methyl-*p*-tyrosine (50–100 mg/kg) which interferes with the synthesis of catecholamines.

5 FLA-63 (25 mg/kg) which is a specific inhibitor of noradrenaline synthesis, was effective in blocking oxolinic acid-related increase of waking and decrease of slow wave sleep.

6 Haloperidol (0.4–0.6 mg/kg) which blocks central dopamine and noradrenaline receptors, reversed oxolinic acid-induced actions on waking and slow wave sleep. Spiroperidol (2–4 mg/kg) which interferes with dopamine and 5-hydroxytryptamine mechanisms, only antagonized the effect of oxolinic acid on light slow wave sleep. REM sleep was further decreased by both neuroleptic agents.

7 γ-Hydroxybutyrate (25–50 mg/kg), which acts as a GABA agonist and amino-oxyacetic acid (20 mg/kg), which considerably increases central GABA levels, were ineffective in blocking oxolinic acid-related disruption of the sleep-wake cycle.

8 Our results suggest that the catecholamines are involved in the arousing effect of oxolinic acid.

Introduction

Oxolinic acid is a quinoline derivative with antimicrobial activity, effectively used in the clinical treatment of urinary tract infections (Madsen & Rhodes, 1971; Ghatikar, 1974). Side-effects observed in patients after therapeutic doses of the compound refer mainly to the central nervous system and consist of insomnia, restlessness and headache (Guyer, 1974; Simon & Chermat, 1977). The compound shows low levels of toxicity in animal studies. Following oral administration, the LD_{50} is 2 g/kg in adult rats (Guyer, 1974). Pharmacological actions after low doses include increased motor activity, irritability and stereotyped behaviour. These effects are observed from approximately 6 mg/kg onwards and last for several hours (Chermat, Kloczko & Simon, 1978; Chermat, Kloczko, Warot & Simon, 1979).

It was our aim to quantify the effects of oxolinic acid on the sleep-wakefulness cycle of the rat. We have also attempted to ascertain the role of catecholamines (dopamine and noradrenaline) and γ-aminobutyric acid (GABA) in the oxolinic acid-induced increase of EEG arousal. To this purpose the effects of oxolinic acid on the sleep-wake cycle were assessed after pretreatment with α-methyl-*p*-tyrosine which interferes with the synthesis of both dopamine and noradrenaline (Andén, Corrodi, Dahlstrom, Fuxe & Hokfelt, 1966) or bis (4-methyl-1-homopiperazinyl-thiocarbonyl) disulphide (FLA-63) which is a specific inhibitor of noradrenaline synthesis (Corrodi, Fuxe, Hamberger & Ljungdahl, 1970); haloperidol or spiroperidol, two dopamine receptor blockers which differ in their relative anti-noradrenaline and anti-5-hydroxytryptamine receptor potencies; amino-oxyacetic acid (AOAA) which exerts a prolonged and powerful depression of GABA-transaminase (GABA-T) activity and considerably increases central GABA levels, or γ-hydroxybutyrate (GHB), a compound chemically related to GABA and assumed to act as a GABA agonist.

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Methods

Male Wistar rats (200–250 g) were implanted chronically with bipolar electrodes (200 μm diameter) on the frontal and occipital cerebral cortices and neck muscles. All the electrodes were soldered to a 7 pin connector (Winchester Electronics, Conn., U.S.A.) cemented to the skull. The animals were housed individually with food and water *ad libitum* and maintained under controlled environmental conditions (12 h light: 12 h dark cycle). Ten days after implantation, when fully recovered, each animal was placed in a dimly lit sound-proof box fitted with a one-way mirror and sleep patterns were recorded. When the animals were habituated to the recording cage and cables, the administration of control solution and drugs was started. Continuous EEG and EMG recordings were made for a period of 5 h. The polygraphic recordings were used for recognizing and quantifying waking (W), drowsiness (SWS1), slow wave sleep (SWS2) and rapid eye movement sleep (REM) as described by Michel, Klein, Jouvet & Valatx (1961) and Lidbrink (1974). Oxolinic acid (Warner-Chilcott) was studied at three dose levels: 8, 16 and 32 mg/kg as base (30.6–122.5 μmol/kg). Next, 32 mg/kg oxolinic acid was injected into animals pretreated with DL-α-methyl-*p*-tyrosine methyl ester hydrochloride (AMPT, Sigma) 50 and 100 mg/kg as salt (0.2–0.4 mmol/kg); FLA-63 (Sigma) 25 mg/kg as base (66 μmol/kg); haloperidol (Janssen) 0.4 and 0.6 mg/kg as base (1–1.6 μmol/kg); spiroperidol (Janssen) 2 and 4 mg/kg as base (5–10 μmol/kg); GHB (sodium salt, Sigma) 25 and 50 mg/kg (0.19–0.38 mmol/kg) or AOAA (Sigma) 20 mg/kg as base (0.18 mmol/kg). Twenty min (haloperidol, spiroperidol, GHB), 60 min (AOAA) or 240 min (AMPT, FLA-63) after the injection of the drugs the animals received the selected dose of oxolinic acid. During control sessions the rats were given AMPT, FLA-63, haloperidol, spiroperidol, GHB, AOAA or the corresponding volumes of solvent. Recordings were always started 20 min after oxolinic acid injection. Oxolinic acid was injected as a suspension in distilled water with Tween 80 added. AMPT, FLA-63, GHB and AOAA were dissolved in 0.9% w/v NaCl solution (saline). Haloperidol and spiroperidol were dissolved in a few drops of glacial acetic acid, the final volume being made up with distilled water and the pH adjusted to 6. Drugs were given intraperitoneally with the exception of FLA-63 which was administered intramuscularly. In order to obtain well-defined effects with the different treatments, at least 5 days were allowed to elapse between experiments. Mean values of the different variables were tested by analysis of variance for dependent samples followed by multiple comparisons using the Scheffé test (Winer, 1962).

Results

Quantitation of the 5 h sessions after oxolinic acid showed marked drug-induced alterations of the sleep-wakefulness cycle. Waking was increased in a dose-related manner, while SWS1, SWS2 and REM were decreased (Table 1). Significant increments in W were observed after the 16 and 32 mg/kg doses. Slow wave sleep decrements only attained significance after the highest dose of the quinoline derivative, while REM time was significantly diminished following the whole range of doses.

As can be seen from Table 2, AMPT (50 to 100 mg/kg) consistently increased SWS2 and decreased SWS1 and W, while REM remained almost unchanged. α-Methyl-*p*-tyrosine counteracted in a dose-related manner the effects of oxolinic acid on W and sleep. In these experiments where the drug was given to animals pretreated with 100 mg/kg AMPT, SWS2 values were considerably larger than control values. On the other hand, W and SWS1 fell to below control levels.

Following FLA-63 (25 mg/kg) no significant changes could be observed on W or sleep (Table 3). Pretreatment with the compound reversed the oxolinic acid-induced increase in W and decrease in SWS1 and SWS2. However, REM depression was not antagonized.

During control sessions haloperidol (0.4 to 0.6 mg/kg) significantly reduced REM while the other variables were modified only slightly (Table 4).

Table 1 Effects of oxolinic acid on some variables of the sleep-wakefulness cycle during 5 h sessions

Treatment	*W*	*SWS1*	*SWS2*	*REM*
I Control	94.2 ± 4.5	31.2 ± 6.3	136.8 ± 6.9	37.8 ± 4.7
II Oxolinic acid 8	108.7 ± 9.5	45.6 ± 17.5	119.0 ± 16.5	26.7 ± 4.6**
16	138.0 ± 8.8**	23.2 ± 5.8	114.5 ± 10.2	24.3 ± 4.2**
32	194.5 ± 17.6**	17.3 ± 2.6	72.7 ± 15.1**	15.5 ± 5.3**

All values are the means (min) ± s.e.mean; $n = 6$. W = waking; SWS1 = drowsiness; SWS2 = slow wave sleep; REM = rapid eye movement sleep.
The drug is placed alongside its dose (mg/kg). Compared to control values: $^{**}P < 0.01$ (Scheffé test).

Table 2 Effects of pretreatment with α-methyl-*p*-tyrosine (AMPT) on the oxolinic acid-induced changes of the sleep-wakefulness cycle

Treatment	*W*	*SWS1*	*SWS2*	*REM*
I Control	91.6± 8.9	54.2±13.5	120.0± 8.6	34.2±3.2
II Oxolinic acid 32	193.2±23.1**	26.0± 4.8	64.8±15.2**	16.0±5.7**
III AMPT 50	96.0±19.7	37.6± 8.4	129.8±20.9	36.6±7.2
100	62.6±13.8	40.4± 8.7	138.4±15.2	38.6±6.9
IV AMPT 50 + oxolinic acid 32	118.4±12.8	42.4± 2.9	113.8± 8.2	25.4±1.8
V AMPT 100 + oxolinic acid 32	71.0±10.0	47.0±10.0	145.6±14.3	36.4±5.7

All values are the means (min) ± s.e.mean; $n = 5$. Abbreviations as in Table 1.
The drugs are placed alongside their doses (mg/kg). Compared to control values: $^{**}P < 0.01$ (Scheffé test).

Table 3 Effects of pretreatment with FLA-63 on the oxolinic acid-induced changes of the sleep-wakefulness cycle

Treatment	*W*	*SWS1*	*SWS2*	*REM*
I Control	93.6± 5.6	32.2± 7.7	135.4± 8.3	38.8±5.7
II Oxolinic acid 32	196.4±21.9**	18.0± 3.1	68.2±17.8**	17.4±6.0**
III FLA-63 25	77.3±14.7	40.5±10.8	150.0±21.0	32.2±2.5
IV FLA-63 25 + oxolinic acid 32	101.6± 8.9	38.4± 3.1	141.0± 9.5	19.0±5.0**

All values are the means (min) ± s.e.mean; $n = 5$. Abbreviations as in Table 1.
The drugs are placed alongside their doses (mg/kg). Compared to control values: $^{**}P < 0.01$ (Scheffé test).

Table 4 Effects of pretreatment with haloperidol on the oxolinic acid-induced changes of the sleep-wakefulness cycle

Treatment	*W*	*SWS1*	*SWS2*	*REM*
I Control	90.8± 5.4	35.7± 7.3	134.7± 6.7	38.8±4.5
II Oxolinic acid 32	193.0±17.8**	20.7± 3.8	69.3±14.3**	17.0±4.8**
III Haloperidol 0.4	95.2±23.0	44.8± 5.9	150.3±21.1	9.7±3.5**
0.6	79.7±26.2	49.2± 7.7	161.7±28.1	9.4±3.6**
IV Haloperidol 0.4 + oxolinic acid 32	122.3±32.5	48.5± 4.8	121.8±31.7	7.4±2.8**
V Haloperidol 0.6 + oxolinic acid 32	101.2±20.1	49.2±11.6	138.3±14.7	11.3±2.7**

All values are the means (min) ± s.e.mean; $n = 6$. Abbreviations as in Table 1.
The drugs are placed alongside their doses (mg/kg). Compared to control values: $^{**}P < 0.01$ (Scheffé test).

Table 5 Effects of pretreatment with spiroperidol on the oxolinic acid-induced changes of the sleep-wakefulness cycle

Treatment	*W*	*SWS1*	*SWS2*	*REM*
I Control	80.5± 7.8	51.1±13.0	132.8±10.9	35.6±4.4
II Oxolinic acid 32	191.5±20.8**	20.6± 5.0**	77.3±14.8**	10.6±4.3**
III Spiroperidol 2	98.3±13.1	42.8± 7.9	153.4±16.3	5.5±2.2**
4	123.8±14.8**	61.6±13.4	108.3±16.3	6.3±3.6**
IV Spiroperidol 2 + oxolinic acid 32	173.5± 9.4**	56.8±12.1	69.0±12.2**	0.7±0.5**
V Spiroperidol 4 + oxolinic acid 32	182.3±16.0**	59.0±12.2	56.0±13.6**	2.7±1.1**

All values are the means (min) ± s.e.mean; $n = 6$. Abbreviations as in Table 1.
The drugs are placed alongside their doses (mg/kg). Compared to control values: $^{**}P < 0.01$ (Scheffé test).

Table 6 Effects of pretreatment with γ-hydroxybutyrate (GHB) on the oxolinic acid-induced changes of the sleep-wakefulness cycle

Treatment	*W*	*SWS1*	*SWS2*	*REM*
I Control	83.5± 6.4	66.7± 9.9	117.3± 7.0	32.5±3.6
II Oxolinic acid 32	200.4±18.2**	25.5± 5.0**	64.8±12.4**	9.3±3.8**
III GHB 25	110.6± 4.9	62.8±12.4	104.3±13.7	22.3±3.6
50	109.1± 5.4	44.8± 8.6*	125.1±10.7	21.0±2.1
IV GHB 25 + oxolinic acid 32	206.3±18.5**	21.1± 3.4**	66.8±14.9*	5.8±2.6**
V GHB 50 + oxolinic acid 32	198.6±16.3**	24.5± 6.3**	65.3±14.5**	11.6±5.2**

All values are the means (min) ± s.e.mean; $n = 6$. Abbreviations as in Table 1.
The drugs are placed alongside their doses (mg/kg). Compared to control values: $*P < 0.05$ (Scheffé test); $**P < 0.01$.

Haloperidol markedly blocked the oxolinic acid-related increase in W. Following the 0.6 mg/kg dose, SWS2 attained values similar to those observed after solvent injection. In contrast, REM showed a further decrease.

Spiroperidol (2 to 4 mg/kg) administration was consistently related to an increase of W, while REM was decreased. SWS2 also was diminished after the largest dose (Table 5). Spiroperidol antagonized the effect of oxolinic acid on SWS1. Conversely, SWS2 and REM showed a further decline. Waking values were similar to those observed during the oxolinic acid sessions.

γ-GHB or AOAA showed no significant effects on the sleep-wake cycle after the range of doses injected (Tables 6 and 7). Pretreatment with GHB was ineffective in modifying the actions of oxolinic acid on the sleep-wakefulness cycle. After treatment with AOAA, SWS2 decrease was slightly antagonized while W, SWS1 and REM remained significantly altered as compared to control.

Discussion

Administration of oxolinic acid modified the rate of cyclic alternation of sleep and wake phases. A significant and dose-related increase of waking EEG was observed. Conversely, SWS and REM were decreased.

As shown by Chermat *et al.* (1978) and Thiébot, Kloczko, Chermat, Simon & Soubrié (1980) oxolinic acid also augments behavioural arousal in rats. According to those authors, the drug consistently induced an increase of locomotor activity and stereotyped behaviour which were antagonized by AMPT, reserpine or pimozide, thus suggesting a facilitatory function of oxolinic acid upon dopaminergic systems.

Present anatomical and pharmacological evidence favours a role for catecholamines as modulators of physiological wakefulness and mediators of the CNS stimulating action of various drugs (Jacobs & Jones, 1978; Ramm, 1979; Monti, 1980). In addition, the central excitatory activity of some other compounds has been ascribed to an inhibition of GABAergic activity (Enna & Maggi, 1979).

The effects of oxolinic acid on W, SWS and REM were antagonized by doses of AMPT which significantly deplete the brain of catecholamines.

FLA-63 also was effective in reversing the oxolinic acid-induced increase of W and decrease of SWS. These results could be related to the depletion of brain noradrenaline. However, the simultaneous increase of 5-HT synthesis by FLA-63 (Johnson & Kim, 1973) tends to indicate a participation of

Table 7 Effects of pretreatment with amino-oxyacetic acid (AOAA) on the oxolinic acid-induced changes of the sleep-wakefulness cycle

Treatment	*W*	*SWS1*	*SWS2*	*REM*
I Control	77.2± 4.8	71.0± 9.4	125.6±10.5	26.2±3.8
II Oxolinic acid 32	178.2±14.6**	25.6± 5.0**	86.6± 8.7*	9.6±3.1**
III AOAA 20	100.2± 4.5	37.2±12.0	145.4±16.9	17.2±5.2
IV AOAA 20 + oxolinic acid 32	179.0±24.4**	19.2± 6.6**	95.0±14.0	6.8±5.4**

All values are the means (min) ± s.e.mean; $n = 5$. Abbreviations as in Table 1.
The drugs are placed alongside their doses (mg/kg). Compared to control values: $*P < 0.05$ (Scheffé test); $**P < 0.01$.

5-hydroxytryptaminergic mechanisms as well. FLA-63 was ineffective in blocking REM depression.

It is well known that changes in the balance between neurotransmitter systems greatly alter REM (King, 1971). As already mentioned, FLA-63 interferes with the oxolinic acid facilitation of noradrenaline mechanisms. In addition, the compound accelerates dopamine synthesis (Nyback, 1971; Andén, Atack & Svensson, 1973). Thus, its inability to antagonize REM depression could be partly related to the increased activity of dopamine containing neurones.

Haloperidol and spiroperidol partially antagonized the oxolinic acid-related changes of the sleep-wake cycle. Haloperidol blocked oxolinic acid effects on W, SWS1 and SWS2. Spiroperidol only antagonized its action on SWS1. Biochemical effects of antipsychotic drugs are not restricted to dopamine receptors. In this regard, studies on turnover of catecholamines in brain have implicated haloperidol as a central antagonist of noradrenaline. Thus, the effectiveness of haloperidol in antagonizing the actions of oxolinic acid on W and SWS could be related to the simultaneous blockade of both dopamine and noradrenaline receptors (Andén, Butcher, Corrodi, Fuxe & Ungerstedt, 1970; Carlsson, 1978).

The pharmacological profile of spiroperidol is probably partly due to 5-hydroxytryptamine (5-HT) receptor blockade, even if the role of dopamine blockade predominates (Leysen, Niemegeers, Tollenaere & Laduron, 1978). Thus, sleep improvement following blockade of dopamine facilitation would be partly counteracted by the simultaneous inhibition of 5-HT receptors. The failure of haloperidol or spiroperidol to reverse the oxolinic acid depression of REM also could be tentatively ascribed to the upset of a dynamic balance between neurotransmitter systems.

GHB which has been assumed to act as a direct or indirect GABA agonist (Andén & Stock, 1973; Carlsson, Biswas & Lindqvist, 1977) was ineffective in reducing the increased amounts of W elicited by oxolinic acid. Similar results were observed after a dose of AOAA which significantly increased central GABA levels (Tzeng & Ho, 1977).

On the basis of the data thus obtained it is suggested that the actions of oxolinic acid on the sleep-wake cycle are due to the facilitation of catecholamine mechanisms.

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