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THE EFFECTS OF NEUROLEPTICS WITH CENTRAL DOPAMINE AND NORADRENALINE RECEPTOR BLOCKING PROPERTIES IN THE L-DOPA AND (+)-AMPHETAMINE-INDUCED WAKING EEG IN THE RAT

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1 A study was carried out in rats (prepared for chronic sleep recording) of the effects of pretreatment with neuroleptics differing in their relative antinoradrenaline and antidopamine receptor potencies, on the L-DOPA and (+)-amphetamine-induced increase in waking EEG.

2 Haloperidol (0.6 mg/kg), which blocks central dopamine and noradrenaline (NA) receptors, reversed the EEG desynchronizing actions of L-DOPA and (+)-amphetamine.

3 Low doses of pimozide (0.4 to 0.8 mg/kg) which block dopamine receptors exclusively, were not able to modify the pharmacologically induced disruption of the sleep-awake cycle. However, at 1 mg/kg, a dose which also produces blockade of NA receptors, pimozide counteracted the increase in waking elicited by (+)-amphetamine.

4 Spiroperidol (1 to 4 mg/kg) which is devoided of NA receptor blocking properties, failed to reverse the L-DOPA or (+)-amphetamine-induced arousal.

5 Our results suggest that the increased waking time observed after the catecholamine agonists is related to an increased availability of NA rather than dopamine.

Introduction

It has been observed that L-dihydroxyphenylalanine (DOPA) and (+)-amphetamine disrupt the sleep-wakefulness cycle of laboratory animals, decreasing slow wave and REM (rapid eye movement) sleep and increasing wakefulness (Altier, Moldes & Monti, 1975; Hartmann & Cravens, 1976).

Studies in rats which had received DOPA indicate that the precursor produces a marked increase in brain dopamine while regional or whole brain levels of noradrenaline (NA) are only slightly enhanced (Romero, Chalmers, Cottman, Lytle & Wurtman, 1972; Benkert, Gluba & Matussek, 1973). On the other hand, amphetamine increases the availability of dopamine and NA to postsynaptic receptors by promoting their release and preventing their uptake (Fuxe & Ungerstedt, 1970).

The results of a variety of experiments suggest that the increased motor activity and stereotyped behaviour induced by DOPA and amphetamine are dependent upon dopaminergic mechanisms (Iversen & Iversen, 1975; Moore, 1977). However, evidence regarding the roles of dopamine and NA in the DOPA or amphetamine-induced cortical desynchrony is scanty. In this connection, Bradley, Candy & Keane (1974) suggest on the basis of studies in 'encéphale isolé' cats, that the desynchronizing actions of amphetamine or DOPA are related to NA.

It was our aim to study further the role of dopamine and NA in the amphetamine and DOPA-induced increase of EEG arousal. To this purpose animals were pretreated with neuroleptics differing in their relative antinoradrenaline and antidopamine-receptor potencies and the effects of DOPA and amphetamine on the sleep-awake cycle were assessed.

Methods

Male Wister rats (250 to 300 g) were implanted chronically with bipolar Nichrome electrodes (200 μm diameter) on the frontal and occipital cerebral cortices and neck muscles. All the electrodes were soldered to a 7 pin connector (Newark Electronics, Chicago, Illinois) 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

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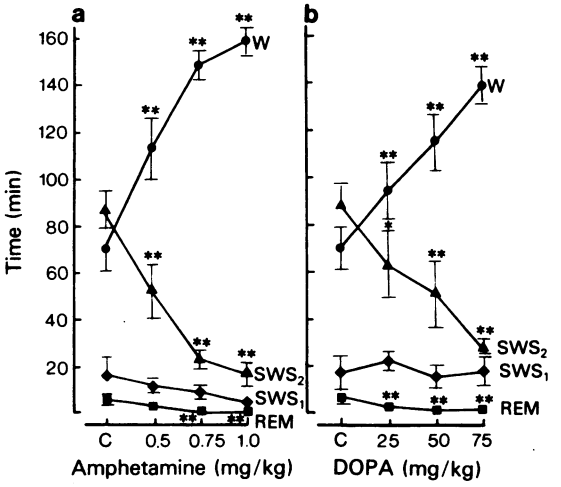

Figure 1 Effects of (+)-amphetamine (a) and L-DOPA (b) on some variables of the sleep-wakefulness cycle during 3 h sessions. C = control; W = awake; SWS1 = drowsiness; SWS2 = slow wave sleep; REM = rapid eye movement sleep; Abscissae: dose in mg/kg; ordinates: time in min. Each point is the mean result of 6 observations; vertical lines show s.e. mean. All comparisons are with control. Differences in mean values were tested by applying the Scheffé test: * $P < 0.05$; ** $P < 0.01$.

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 3 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).

The catecholamine agonists were studied at three dose levels: (1) L-DOPA (Roche) 25, 50 and 75 mg/kg. Benserazide was always given in a dose of 50 mg/kg, 30 min before DOPA, in order to block extracerebral decarboxylase. (2) (+)-Amphetamine sulphate (S, K & F) 0.5, 0.75 and 1 mg/kg as salt. During a second stage 75 mg/kg DOPA or 1 mg/kg amphetamine were injected into animals pretreated with haloperidol (Janssen) 0.2, 0.4 and 0.6 mg/kg (0.53 to 1.6 μmol/kg); pimozide (Janssen) 0.4, 0.6, 0.8 and 1 mg/kg (0.87 to 2.2 μmol/kg) or spiroperidol (Janssen) 1, 2 and 4 mg/kg (1.9 to 7.6 μmol/kg). Thirty min (haloperidol, spiroperidol) or 180 min (pimozide) after the injection of the antagonists the animals received the selected doses of DOPA or amphetamine.

During control sessions the rats received haloperidol, pimozide, spiroperidol or the corresponding volumes of solvent.

Recordings were always started 10 min after the last drug injection. DOPA was injected as a suspen-

Table 1 Effects of pretreatment with haloperidol on the (+)-amphetamine and L-DOPA-induced changes of the sleep-wakefulness cycle

Treatment	*Dose (mg/kg)*	*W*	*SWS1*	*SWS2*	*REM*
I Control		69 ± 7	13 ± 1	88 ± 6	10 ± 2
II Haloperidol	0.2	48 ± 6*	23 ± 6	101 ± 9	8 ± 4
	0.4	44 ± 9*	23 ± 4	112 ± 10*	1 ± 1*
	0.6	33 ± 7**	22 ± 6	121 ± 10**	0**
III Amphetamine	1	158 ± 6**	5 ± 1	17 ± 5**	0**
IV Haloperidol + amphetamine	0.2 1	114 ± 10**	15 ± 2	51 ± 8**	0**
V Haloperidol + amphetamine	0.4 1	102 ± 12*	27 ± 7	51 ± 7**	0**
VI Haloperidol + amphetamine	0.6 1	51 ± 13*	39 ± 8**	89 ± 18	1 ± 1*
VII DOPA	75	138 ± 8**	17 ± 6	25 ± 5**	0**
VIII Haloperidol + DOPA	0.2 75	96 ± 28	33 ± 11	50 ± 18**	1 ± 1*
IX Haloperidol + DOPA	0.4 75	86 ± 18	31 ± 5	63 ± 15	0**
X Haloperidol + DOPA	0.6 75	65 ± 13	32 ± 5	83 ± 16	0**

All values are the means in min ± s.e. mean; $n = 6$.
Compared to control values: * $P < 0.05$; ** $P < 0.01$ (Scheffé test).

sion in distilled water with Tween 80 added. Amphetamine and benserazide were dissolved in 0.9% w/v NaCl solution (saline). Haloperidol, pimozide and spiroperidol were dissolved in a few drops of glacial acetic acid, the final volume being made up with saline and the pH adjusted to 6. In order to obtain well-defined effects with the different treatments, at least 1 week was allowed to elapse between experiments.

Mean values of the several variables were tested by analysis of variance for dependent samples followed by multiple comparisons using the Scheffé test (Winer, 1962).

Results

Quantitation of the 3 h sessions after DOPA or amphetamine showed significant drug-induced alterations of the sleep-wakefulness cycle. Waking was increased in a dose-related manner, while SWS2 and REM were decreased (Figure 1). REM was abolished after the highest doses of DOPA or amphetamine.

As can be seen from Table 1, haloperidol (0.4 to 0.6 mg/kg) significantly augmented SWS2 while W and REM were diminished; in addition, it counteracted in a dose-related manner the effects of amphetamine and DOPA on some variables of the sleep-awake cycle. In the DOPA experiments, the 0.6 mg/kg dose of haloperidol returned W and SWS2 to control levels. In amphetamine experiments, the highest dose of haloperidol significantly decreased W and increased SWS1 as compared to control. SWS2 attained values similar to those observed after solvent injection. Haloperidol did not antagonize the DOPA or amphetamine-induced depression of REM (Table 1).

Pimozide significantly decreased W, while SWS and REM were only slightly modified (Table 2). The compound was ineffective in reversing the DOPA-induced increase in waking EEG. On the other hand, the highest dose of pimozide prevented the increase in W becoming significantly different from control values after amphetamine (Table 2).

Following spiroperidol, no significant changes could be observed in total W or SWS2 as compared to control. However, REM was significantly decreased while SWS1 was increased (Table 3). Spiroperidol was ineffective in antagonizing the increased

Table 2 Effects of pretreatment with pimozide on the (+)-amphetamine and L-DOPA-induced changes of the sleep-wakefulness cycle

Treatment	*Dose (mg/kg)*	*W*	*SWS1*	*SWS2*	*REM*
I Control		69 ± 7	13 ± 1	88 ± 6	10 ± 2
II Pimozide	0.4	42 ± 3**	20 ± 3	102 ± 2	16 ± 1
	0.6	41 ± 7**	19 ± 6	103 ± 9	17 ± 3
	0.8	46 ± 5*	18 ± 8	102 ± 9	14 ± 2
	1	49 ± 6*	30 ± 8*	91 ± 10	10 ± 3
III Amphetamine	1	158 ± 6**	5 ± 1	17 ± 5**	0**
IV Pimozide + amphetamine	0.4 1	114 ± 10**	10 ± 2	55 ± 9**	1 ± 1*
V Pimozide + amphetamine	0.6 1	122 ± 9**	13 ± 2	45 ± 8**	0**
VI Pimozide + amphetamine	0.8 1	107 ± 13*	20 ± 4	53 ± 9**	1 ± 1*
VII Pimozide + amphetamine	1 1	96 ± 7	10 ± 1	73 ± 7	1 ± 1*
VIII DOPA	75	138 ± 8**	17 ± 6	25 ± 5**	0**
IX Pimozide + DOPA	0.6 75	135 ± 9**	18 ± 4	27 ± 7**	0**
X Pimozide + DOPA	0.8 75	125 ± 11**	19 ± 3	36 ± 10**	0**
XI Pimozide + DOPA	1 75	113 ± 15**	20 ± 4	47 ± 14**	0**

All values are the means in min ± s.e. mean; $n = 6$.
Compared to control values: * $P < 0.05$; ** $P < 0.01$ (Scheffé test).

EEG arousal caused by amphetamine or DOPA (Table 3).

Discussion

In order to determine the relative influence of dopamine and NA in the DOPA and amphetamine-induced increase in waking EEG, animals were pretreated with neuroleptics which either selectively block dopamine receptors or simultaneously act on both dopamine and NA synaptic sites.

Haloperidol, which at a dose of 0.6 mg (1.6 μmol)/kg produces a marked blockade of dopamine receptors together with smaller although significant effects on NA receptors (Andén, Butcher, Corrodi, Fuxe & Ungerstedt, 1970; Carlsson, 1978) proved to be the most effective in reversing the EEG desynchronizing actions of DOPA or amphetamine. Interestingly enough, the REM depression was not reversed, which suggests that the effects of the CA agonists on this type of sleep are related to synaptic sites other than those influenced by haloperidol.

Doses of 0.4 to 0.8 mg/kg pimozide exclusively block dopamine receptors. However, at 1 mg (2.2 μmol)/kg it also blocks NA receptors (Andén, Corrodi & Fuxe, 1972). Correspondingly, antagonism of amphetamine-induced increase of waking time was only obtained after the 1 mg/kg dose. Pimozide was shown to be about 1.4 times less potent (on a molar basis) than haloperidol as regards its NA receptor blocking activity. Most probably, higher doses of pimozide would have also been effective in antagonizing the DOPA-induced disruption of the sleep-awake cycle.

According to results obtained in turnover studies, spiroperidol blocks only central dopamine receptors (Carlsson, 1978). In contrast to haloperidol and pimozide, spiroperidol was ineffective in antagonizing the amphetamine and DOPA-induced increase of W. Thus, our results tend to suggest that only CA antagonists sharing NA receptor blocking properties are able to antagonize the DOPA or amphetamine arousing effects. In this connection, the increase in waking time observed after the administration of amphetamine or DOPA is apparently related to an increased availability of NA rather than dopamine.

Using different experimental approaches, several authors have also related the presence of NA in the brain to electrocortical desynchronization. Thus, Lidbrink, Corrodi, Fuxe & Olson (1972) found that FLA-63, which inhibits the conversion of dopamine to NA decreases W time, while Key (1975) induced a state of tonic electrocortical desynchronization after perfusing NA into the pontine and mesencephalic reticular formation.

Thus, our results give further support to previous work suggesting a noradrenergic component in the arousal system.

Table 3 Effects of pretreatment with spiroperidol on the (+)-amphetamine and L-DOPA-induced changes of the sleep-wakefulness cycle

Treatment	*Dose (mg/kg)*	*W*	*SWS1*	*SWS2*	*REM*
I Control		64 ± 12	14 ± 4	91 ± 9	11 ± 2
II Spiroperidol	1	63 ± 13	32 ± 7**	84 ± 16	1 ± 1**
	2	74 ± 10	19 ± 4	86 ± 10	1 ± 1**
	4	79 ± 10	28 ± 4*	73 ± 12	0**
III Amphetamine	1	150 ± 5**	9 ± 3	20 ± 4**	1 ± 1**
IV Spiroperidol + amphetamine	1 1	111 ± 9**	16 ± 4	53 ± 10**	0**
V Spiroperidol + amphetamine	2 1	118 ± 9**	16 ± 4	46 ± 8**	0**
VI Spiroperidol + amphetamine	4 1	138 ± 8**	23 ± 6	19 ± 6**	0**
VII DOPA	75	158 ± 5**	9 ± 4	13 ± 3**	0**
VIII Spiroperidol + DOPA	1 75	150 ± 7**	13 ± 2	17 ± 6**	0**
IX Spiroperidol + DOPA	2 75	150 ± 14**	14 ± 7	16 ± 8**	0**
X Spiroperidol + DOPA	4 75	161 ± 18**	14 ± 5	5 ± 3*	0**

All values are the means in min ± s.e. mean; $n = 6$.
Compared to control values: * $P < 0.05$; ** $P < 0.01$ (Scheffé test).

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