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THE ACTIONS OF DIHYDROXYPHENYLALANINE AND DIHYDROXYPHENYLSERINE ON THE SLEEP-WAKEFULNESS CYCLE OF THE RAT AFTER PERIPHERAL DECARBOXYLASE INHIBITION

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1 The actions of dihydroxyphenylalanine (DOPA) and dihydroxyphenylserine (DOPS) were assessed on the sleep-wakefulness cycle of male Wistar rats.

2 In comparative studies the extracerebral decarboxylase was inhibited with serine-trihydroxybenzylhydrazide (RO 4-4602) before injection of DOPA or DOPS.

3 DOPA (80-160 mg/kg, i.p.) with or without previous inhibition of the peripheral decarboxylase gave rise to an initial significant increase of slow wave activity, which may be related to a release of 5-hydroxytryptamine.

4 During the subsequent 8 h sessions, DOPA significantly decreased slow wave sleep and rapid eye movement sleep (REM) and increased wakefulness.

5 DOPS (80-160 mg/kg, i.p.) did not significantly modify the sleep-wakefulness cycle apart from a decrease of the latency for the first REM episode after 160 mg/kg in the RO 4-4602 pretreated animals.

Introduction

There is at present evidence indicating that catecholamines may be involved in arousal and rapid eye movement sleep (REM), while indoleamines may be related to slow wave sleep (SWS) (Torda, 1968; Jouvet, 1969). Part of this evidence has been obtained by the use of precursors of these amines, including L-dihydroxyphenylalanine (DOPA), DL-dihydroxyphenylserine (DOPS) and L-5-hydroxytryptophan (5-HTP). Since these compounds cross the blood-brain barrier only to a limited extent (Constantinidis, de la Torre, Tissot & Geissbulher, 1969), controversies have arisen as to the origin of the central actions seen after their administration. In this connection, the pronounced autonomic symptoms produced by the injection of DOPA, ascribable to the actions of newly formed catecholamines in the periphery, would be partly responsible for the state of behavioural arousal simultaneously developed (Butcher & Engel, 1969). There is also controversy as to the action of the precursors on the EEG, especially with respect to DOPA, which has been most extensively used. While some authors describe a cortical desynchronization after low doses of DOPA, others emphasize that desynchronization cannot be observed until doses in the lethal range are approached (Harner & Dorman, 1969; Thut & Rech, 1972).

Discrepancies in results could arise not only because of differences in the species examined, different dose ranges and routes of administration, but also as a result of differences in the time of observation, since the biochemical changes induced by the precursor (Ng, Chase, Colburn & Kopin, 1972) could have a temporal sequence giving rise to different EEG patterns.

There is also uncertainty as to whether the EEG effects are due to dopaminergic and/or noradrenergic mechanisms (Lidbrink, Corrodi, Fuxe & Olson, 1972).

It was our aim to study the actions of two catecholamine precursors upon the EEG and the sleep-wakefulness cycle of the rat, of which one, DOPA, was found to produce a marked increase in brain dopamine (Bartholini, Da Prada & Pletscher, 1968), while the other, DOPS, selectively increased noradrenaline (NA) in animal brain (Blaschko, Burn & Langemann, 1950).

In order to exclude the actions of peripherally formed catecholamines, in some experiments extracerebral decarboxylase was inhibited before the injection of the precursors.

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Methods

Six male Wistar rats weighing 180-200 g were used in the EEG studies. They were housed individually and maintained under controlled environmental conditions, with a cycle of 12 h light-12 h darkness (light cycle was from 9 h 00 min to 21 h 00 min). All the animals were chronically implanted with bipolar Nichrome electrodes (200 μm diameter) in the frontal and occipital cerebral cortices, dorsal hippocampus (A 3.0, L 2.0, H +2.0; König & Klippel, 1963) and neck muscles. All the electrodes were wired to a small connector fixed to the skull with dental acrylic. Histological verification of the position of the electrodes was carried out at the end of the experiments.

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 with a Grass 7 polygraph. When the animals were fully adapted to their new environment as judged by the consistency of their sleep-wakefulness cycles, solvent and drug administration were started. All records were scored for waking, SWS and REM, according to standard criteria.

Three sets of experiments were carried out in a random order:

Experiment 1 DOPA and DOPS were studied at 2 dose levels, 80 and 160 mg/kg.

Experiment 2 A dopa-decarboxylase inhibitor, RO 4-4602 (DL-serine 2-(2,3,4-trihydroxybenzylhydrazide hydrochloride) was administered in a dose of 50 mg/kg, in order to block only extracerebral decarboxylase.

Experiment 3 RO 4-4602 was given followed 30 min later by DOPA or DOPS. During control experiments, the rats received the corresponding volumes of solvent.

Drugs were administered as aqueous solutions or suspensions prepared with distilled water. All the injections were given intraperitoneally and recordings were started immediately after the last injection, although scoring of the 8 h sessions began 20 min later. At least one week was allowed to elapse between experiments and each treatment was given to each animal twice.

Differences in 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

Following the administration of DOPA, the animals showed behavioural quiescence. No loss of righting reflex or postural changes could be observed. About 15-20 min after the injection of the catecholamine precursor, marked autonomic symptoms including salivation, piloerection, exophthalmus and increased respiratory rate became evident, lasting about 60-90 minutes. When DOPA was preceded by RO 4-4602, no autonomic signs could be observed, the main effects being stereotyped movements of the head and forepaws which were more pronounced after the dose of 160 mg/kg DOPA and lasted 120-180 minutes.

After DOPA administration with or without the decarboxylase inhibitor, a significant increase of slow wave EEG activity compared with the effect of solvent was seen during the first 20 min of continuous recording (Table 1). Moreover, there

Table 1 Amounts of slow wave (synchronized) and fast (desynchronized) EEG activity during the first 20 min following L-dihydroxyphenylalanine (DOPA) or DL-dihydroxyphenylserine (DOPS) and pretreatment with RO 4-4602

Treatment (mg/kg)	*EEG* *Slow wave activity (s ± s.e. mean)*	*Fast activity (s ± s.e. mean)*	P*
Control	43 ± 24	1157 ± 24	–
DOPA (80)	481 ± 98	719 ± 98	<0.01
DOPA (160)	373 ± 96	827 ± 96	<0.05
RO (50 + DOPA·(80)	300 ± 77	900 ± 77	<0.05
RO (50) + DOPA (160)	330 ± 47	870 ± 47	<0.01
DOPS (80)	196 ± 108	1004 ± 108	NS
DOPS (160)	113 ± 50	1087 ± 50	NS
RO (50) + DOPS (80)	148 ± 87	1052 ± 87	NS
RO (50) + DOPS (160)	268 ± 78	932 ± 78	NS

* All comparisons are with control. Differences in mean values were tested by applying the Scheffé test.

were no significant differences in the amount of slow wave activity following different DOPA treatments.

The EEG synchronization contrasted with the animal's behaviour, standing with open eyes. Subsequently, the EEG pattern shifted to one of continuous desynchronization, the duration varying according to the treatment involved.

Quantitation of the 8 h sessions showed significant drug-induced alterations of the sleep-wakefulness cycle. DOPA (80 mg/kg) increased wakefulness and decreased REM. Wakefulness was augmented mainly during the first 2 h of recording; REM was abolished during the first hour, thereafter increasing steadily and reaching control levels at the fourth hour (Figure 1; Table 2).

Doubling of the dose of DOPA produced a further increase of waking, while SWS and REM time were simultaneously decreased. Waking and SWS attained control values at the fourth hour, while REM returned to control levels 2 h later (Figure 1; Table 2).

Following a dose of 50 mg/kg RO 4-4602, no significant changes could be observed (Table 2). After pretreatment with RO 4-4602, a marked increase of the effects of 80 and 160 mg/kg DOPA on the sleep-wakefulness cycle was observed. Wakefulness became almost continuous during the first 120-180 min and SWS fully recovered only after the fourth and fifth hour following the low and high doses, respectively. REM which was

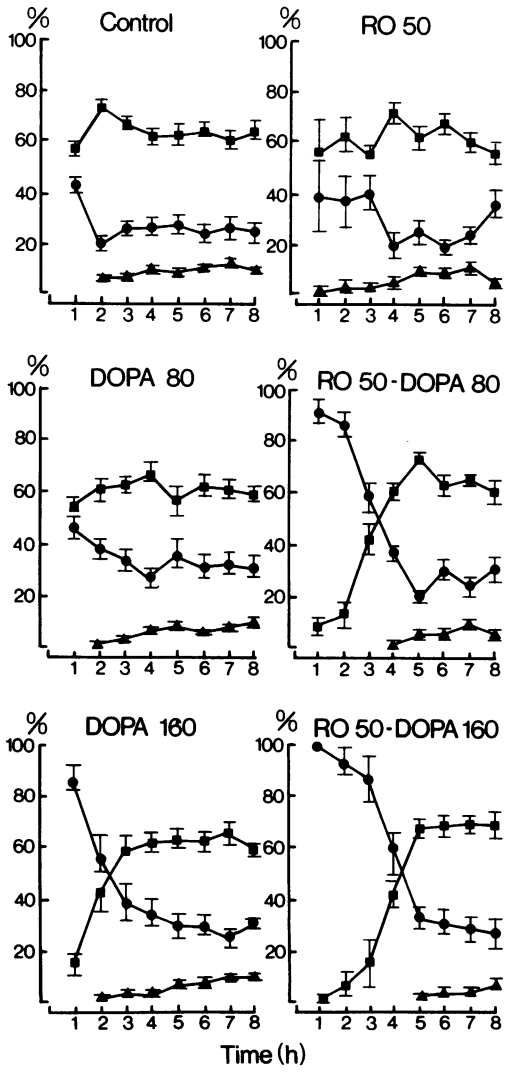

Figure 1 Percentages of waking (•), slow wave sleep (▪) and rapid eye movement sleep (▲) during the 8 h sessions after L-dihydroxyphenylalanine (DOPA) administration and pretreatment with RO 4-4602. Abscissae: time in hours. Ordinates: percentage (±s.e. mean) of behavioural stage according to EEG criteria. Doses of drugs in mg/kg.

Table 2 Effects of L-dihydroxyphenylalanine (DOPA) and pretreatment with RO 4-4602 on some variables of the sleep-wakefulness cycle during 8 h sessions starting 20 min after the last injection

Treatment (mg/kg)	*Wakefulness (min ± s.e. mean)*	*Slow wave sleep (min ± s.e. mean)*	*REM sleep (min ± s.e. mean)*
Control	137 ± 14	301 ± 13	42 ± 4
DOPA (80)	167 ± 13	286 ± 12	27 ± 2**
DOPA (160)	215 ± 21**	243 ± 19**	22 ± 3**
RO (50)	145 ± 15	299 ± 13	36 ± 5
RO (50) + DOPA (80)	225 ± 13**	236 ± 9**	19 ± 5**
RO (50) + DOPA (160)	274 ± 14**	199 ± 12**	7 ± 2**

Differences in mean values were compared to control values and tested for significance by applying the Scheffé test.
** $P < 0.01$.

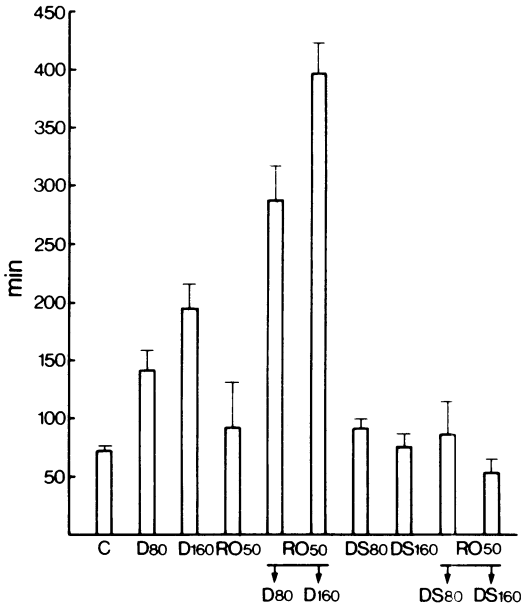

Figure 2 Latencies for the appearance of the first rapid eye movement sleep episode following L-dihydroxyphenylalanine (D) or DL-dihydroxyphenylserine (DS) administration and pretreatment with RO 4-4602. Abscissae: pharmacological treatments. The numbers adjacent to the letters indicate mg/kg of drug administered.

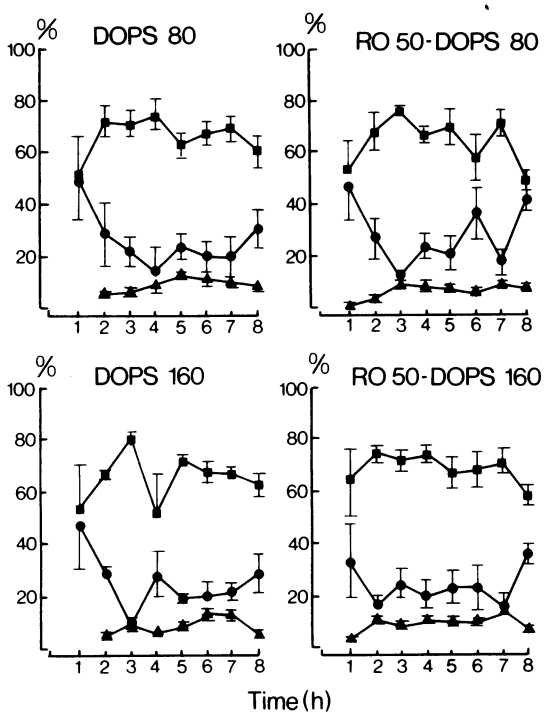

Figure 3 Percentages of waking (•), slow wave sleep (■) and rapid eye movement sleep (▲) during the 8 h sessions after DL-dihydroxyphenylserine (DOPS) administration and pretreatment with RO 4-4602. Abscissae: time in hours. Ordinates: percentage (±s.e. mean) of behavioural stage according to EEG criteria. Doses of drugs in mg/kg.

almost absent during the first 3-4 h after the smallest dose of the precursor, reached control values at the seventh hour. REM values were below control values throughout the recovery period (Figure 1).

The latency for the appearance of the first REM period was significantly increased after DOPA ($P < 0.01$) in a dose-related manner. There was a further increase in this effect of DOPA after inhibition of peripheral dopa-decarboxylase (Figure 2) although RO 4-4602 alone had no direct effect.

DOPS in doses of 80 and 160 mg/kg with or without decarboxylase inhibition did not induce behavioural alterations. While asleep, the animals always exhibited normal postures. Although EEG synchronization was augmented during the first 20 min following DOPS, the increase did not reach significance (Table 1). Slow wave sleep values after 160 mg/kg DOPS and 50 mg/kg RO 4-4602 plus

Table 3 Effects of DL-dihydroxyphenylserine (DOPS) and pretreatment with RO 4-4602 on some variables of the sleep-wakefulness cycle during 8 h sessions starting 20 min after the last injection

Treatment (mg/kg)	*Wakefulness (min ± s.e. mean)*	*Slow wave sleep (min ± s.e. mean)*	*REM sleep (min ± s.e. mean)*
Control	137 ± 14	301 ± 13	42 ± 4
DOPS (80)	122 ± 25	318 ± 23	40 ± 4
DOPS (160)	121 ± 20	322 ± 20	37 ± 6
RO (50) + DOPS (80)	141 ± 18	306 ± 14	33 ± 4
RO (50) + DOPS (160)	113 ± 22	325 ± 16	42 ± 6

Differences in mean values were not significant.

80 mg/kg DOPS were much higher than control values during the third hour, but no significant changes of total wakefulness, SWS and REM time were observed during the 8 h sessions (Table 3; Figure 3). However, the latency for the first REM episode diminished significantly ($P < 0.01$) after 160 mg/kg DOPS in the RO 4-4602 pretreated animals (Figure 2).

Discussion

The administration of DOPA modified the spontaneous behaviour of the rats as well as their rate of cyclic alternation of sleep and wake phases. When they received DOPA without previous inhibition of the peripheral decarboxylase, a decrease of ongoing motor activity and symptoms of increased autonomic activity became evident. Both have been related by Carlsson (1965) to catecholamines formed from DOPA at different peripheral sites. When the decarboxylation of DOPA at extracerebral sites was prevented, the behavioural modifications induced by the precursor had an opposite nature; that is, increased spontaneous motor activity together with stereotyped movements appeared. Butcher & Engel (1969) relate them to newly formed catecholamines (mainly dopamine) at different central sites.

During the first 20 min following the injection of DOPA with or without a peripheral decarboxylase inhibitor, a significant increase of slow wave activity was observed in the EEG. In spite of the electrocortical synchronization, the animals were aroused; that is, a dissociation between EEG and behaviour took place. Synchronization of the EEG followed by a prolonged waking state was also reported by Gaillard, Friedli & Tissot (1973) after intravenous infusion of DOPA in rabbits pretreated with a decarboxylase inhibitor. However, previous studies dealing with the actions of DOPA on the rat EEG, where the precursor was administered intraperitoneally, failed to show an initial synchronization, because the recordings were started 20-25 min after drug injection, when the EEG pattern had already shifted to one of fast and low voltage waves (Monnier, 1960; Thut & Rech, 1972).

There is evidence linking 5-hydroxytryptamine (5-HT) with the EEG components of SWS (Jouvet, 1969). Further, Ng, Chase, Colburn & Kopin (1970) found that DOPA markedly increased the efflux of 5-HT from rat brain slices, this action being contingent on the decarboxylation of DOPA to dopamine. In agreement with these *in vitro* results is the finding by Karobath, Diaz & Huttunen (1971) that intraperitoneal injection of DOPA decreases 5-HT levels in rat brain. Hence, it could be suggested that the initial synchronization after DOPA administration is related to the release of 5-HT.

The initial stage of increased EEG synchronization was later substituted by another of continuous EEG desynchronization, the duration of which was related to the dose of DOPA injected. After 80-160 mg/kg DOPA, waking time exceeded that of SWS during the first 60-120 minutes. When the peripheral decarboxylase was inhibited prior to DOPA injection, wakefulness was almost continuous during the first 120-180 min also surpassing SWS numerically during the first 180-240 minutes. Values for the entire 8 h sessions varied in a dose-related manner and inhibition of the peripheral decarboxylase, while increasing DOPA effects on the sleep-wakefulness cycle, failed to alter this relationship. REM followed a different course, which was characterized by an initial suppression lasting 60-240 min according to the treatment involved. Recovery was gradual with no further rebound.

The measurement of catecholamine levels in the central nervous system after DOPA injection by Butcher & Engel (1969), Everett & Borcherding (1970) and Benkert, Gluba & Matussek (1973) showed that the precursor produced a marked increase in brain dopamine, no change in NA and a decrease in 5-HT. When given to RO 4-4602 pretreated animals, the drug produced a further increase of central dopamine levels, while the NA content was not modified. However, Romero, Chalmers, Cottman, Lytle & Wurtman (1972) found that although total concentrations of NA are not modified after DOPA, the telencephalon, hypothalamus and cerebellum contain significantly more NA. It could be suggested, therefore, that both total dopamine and regional NA increases are related to the disruption of the sleep-wakefulness cycle found by us after DOPA administration.

DOPS did not significantly modify the sleep-wakefulness cycle apart from a decrease of the latency for the first REM episode after 160 mg/kg in the RO 4-4602 pretreated animals. In contrast to DOPA, blockade of peripheral decarboxylase did not increase DOPS effects on waking, SWS and REM. Havlicek (1967), using a different approach, found that intravenous infusion of 500 mg/kg DOPS was followed 2 h later by a very slight increase of REM together with an unusual kind of SWS, characterized by the fact that the animals were lying on their sides.

It is known that doses of DOPS in the range used by us produce a relatively slight increase in cerebral NA, while pretreatment with RO 4-4602 abolishes the increase (Bartholini, Constantinidis, Tissot & Pletscher, 1971; Benkert *et al.*, 1973).

These facts, most probably related to the low affinity of DOPS for the decarboxylase and to the inhibition by RO 4-4602 of DOPS entry into the brain (Holtz, 1959; Bartholini *et al.*, 1971), could tentatively explain the low amount of initial synchronization as well as the absence of significant changes of the sleep-wakefulness cycle variables after DOPS. Therefore, in order to determine the effects of a selective rise of NA on the sleep-wakefulness cycle, more suitable methods are needed.

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