ROLE OF ACTH ON THE EFFECT OF MEDROXYPROGESTERONE IN BRAIN STEM SEROTONIN.

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Summary: Pregnenolone, medroxyprogesterone (MPA) and melengestrol (MLA) caused a significant decrease of brain stem serotonin (5-HT) in the adult female rat, without modifying the content of 5-hydroxy indoleacetic acid (5-HIAA). Only MPA and MLA increased the liver tryptophan pyrrolase (LTP) activity. Chlormadinone, in spite of its similar chemical structure, had no effect. Both total and free plasma tryptophan were not modified, except the latter which decreased when MPA was injected. The steroids herein studied had no effect on brain stem tryptophan and monoamino oxidase (MAO) system activity. The effect of MPA on 5-HT was antagonized in fasting, adrenalectomized and normal rats injected with ACTH. These results suggest that ACTH would participate in the effect of MPA on brain stem 5-HT.

Introduction.

Previous studies (Algeri et al. 1977) demonstrated that medroxyprogesterone acetate (MPA) injected to female rats, diminished

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the serotonin content in brain stem without modifying that of 5-hydroxy indoleacetic acid (5-HIAA), its principal metabolite. Several authors suggested the participation of peripheric factors in the regulation of serotonin biosynthesis (Hamon & Glovinsky, 1974), among them: liver tryptophan pyrrolase (LTP)(Curzon & Green, 1969; Green et al.,1975; Lee & Baltz, 1962; Hiller et al.1975; Badawy,1977), plasmatic levels of non-esterified free fatty acids (NEFA) (Fernstrom & Wurtman, 1973; Curzon & Knott, 1975; Hutson et al.,1976), glucose (Fernstrom & Wurtman, 1972), free and bound tryptophan (Perez-Cruet, 1972; rev.Paoletti et al., 1975), content of this aminoacid and its competence with others (Fernstrom & Wurtman, 1972). Probably the metabolites of tryptophan by effect of LTP, during stress (kynurenine and its metabolites), are involved in a disturbance of the circulation (according to Lapin, 1976). Certain steroids (hydrocortisone) and kynurenine (Curzon & Green, 1971) also modify brain 5-HT. In the changes of brain 5-HT, several factors must be considered: "time of observation, age of animals and hydrocortisone preparation used" (Green et al., 1975).

Holub et al. (1961) proved that a single injection of 6-CH_3-17-acetoxy-progesterone to rats inhibited ACTH release in acute stress. Treatment with MPA also caused a decrease in adrenal weight and corticosterone levels in rats (Givner and Rochefort, 1972). Preliminary investigations (Izquierdo et al., 1975; Savi ni et al., 1976) demonstrated that MPA acetate injected to female rats decreased the 5-HT content in brain stem without modifying the content of 5-HIAA. The balance between free and bound tryptophan would play an important role in the free tryptophan availability for the uptake into central serotoninergic neurons

(Bloxam et al., 1974). MPA, a progestational agent, would be an inhibitor of adenohypophyseal trophins liberation (Fernstrom et al.,1971; Sadeghi-Nejad et al., 1971; Sherman et al.,1971; Baker et al.,1972; Jones et al.,1974; Nugent et al.,1975).

The present investigation tends to elucidate the mechanism by which MPA lowered 5-HT/5-HIAA ratio in the brain area studied and the possible role of serotoninergic mechanisms (Vernikos-Danellis, 1973; Cocchi, et al., 1974) on hypophyseal regulation in the female rat. The participation of MAO system was determined with the same purpose and, also the effects of some steroids were studied in order to find a relationship between chemical structure and activity.

Material and methods.

Three hundred sixty one female virgin rats, Wistar strain, were used, weighing 110-140 g and approximately of 45 days of age. They were fed Purine and tap water ad libitum, maintained at 22-24° C and exposed to light from 7 am to 7 pm. They were housed 4 per box and after a 6-days stay in the same room they were sacrificed. The animals under fasting were deprived of food 24 hours before being killed, but received water.

Fifteen rats were adrenalectomized and maintained during 7 days with water ad libitum with addition of 9‰ NaCl and vitamins. Only those animals showing no accessory adrenals and negligible values of plasma corticosterone's levels at post-mortem examination were used.

Ten sham-operated rats were kept as controls.

Drugs employed:

Medroxyprogesterone acetate, aqueous susp.(The Upjohn Co.,Kalamazoo);

Melengestrol acetate, aqueous susp.(The Upjohn Co., Kalamazoo);

Chlormadinone, aqueous susp. (G.Ramon Laboratories);Pregnenolone, aqueous susp. (Sigma Co); -methyl pregnenolone, aqueous susp. (Sigma Co); DOCA, acetate, oil sol, 17-α-hydroxyprogesterone, oil sol. (Schering, Germany); Acetoxi-progesterone, aqueous susp. (Gador laboratories) and ACTH(Synacthen) aqueous susp. (Ciba-Geigy).

All the drugs except ACTH were injected subcutaneously (20mg/kg) at 12 pm, eight hours before killing the animals. ACTH was injected subcutaneously (20 IU/rat and 10 IU/rat) 24 and 8 hours before death. Controls were injected with respective vehicles (2ml/kg). In all cases, statistical analysis was performed using Student's "t" test. Rats were sacrificed using a guillotine and blood was collected in heparinized tubes and immediately the brain stem was dissected on Petri dishes with ice. The selected brain area had the upper mammillary corps in front and the medulla in the rear.

Determinations in liver tissue were performed in ± 100 mg using a glass homogenizer with Teflon pestle.

The following parameters were measured: 1) LTP: Knox and Auerbach (1955); 2) glycemia: glucose oxidase (Kit; Boehringer Mannheim, Germany); 3) NEFA: Ducombe (modified) (Kit; Boehringer Mannheim, Germany); 4) Plasmatic (free and bound) and brain Trp: Denckla and Dewey (1967); 5) Plasma corticosterone: Guillemin (modified) (1959); 6) MAO: Michel Krajl (1965) and 7) 5-HT and 5-HIAA: Curzon and Green (1970).

Results.

The results obtained with the steroids studies are summarized in Tables I to VI.

TABLE I

Effects on LTP activity

	controls	treated	P
MPA	8.4 ± 0.9 (12)	14.6 ± 1.4 (12)	<0.005
MLA	11.0 ± 0.9 (14)	22.6 ± 1.8 (14)	<0.001
Pregnenolone	10.3 ± 0.1 (4)	8.6 ± 0.9 (4)	
αCH_3-Pregnenolone	13.5 ± 2.8 (4)	15.9 ± 1.4 (4)	
17α HO-Progesterone	8.0 ± 0.7 (4)	9.0 ± 0.8 (4)	
Chlormadinone	9.4 ± 0.6 (7)	10.3 ± 1.3 (6)	
DOCA	10.6 ± 1.0 (4)	12.3 ± 1.4 (4)	
Acetoxy progesterone	9.0 ± 1.2 (4)	11.5 ± 1.0 (4)	

$\bar{X}$ ± SEM: results expressed in µmol kynurenine/g dry tissue/h.

In brackets: number of animals.

TABLE II

Effects on total and free plasma and brain stem Trp

		Plasma Trp µg/ml. plasma			Brain Trp µg/g fresh tissue
		Total	Free		
MPA	C	30.31 ± 1.38(8)	1.52 ± 0.07(7)	P<0.01	2.86 ± 0.16(8)
	T	28.77 ± 1.83(8)	1.24 ± 0.07(8)		2.91 ± 0.16(8)
MLA	C	26.97 ± 1.36(8)	1.32 ± 0.09(8)		2.12 ± 0.15(7)
	T	26.40 ± 1.36(8)	1.09 ± 0.08(7)		1.95 ± 0.08(8)
Chlormadinone	C	34.5 ± 1.00(7)	1.79 ± 0.40(5)		1.72 ± 0.18(8)
	T	33.1 ± 1.25(8)	1.50 ± 0.21(8)		1.52 ± 0.09(8)
Pregnenolone	C	23.19 ± 2.64(4)	0.90 ± 0.02(3)		2.15 ± 0.26(3)
	T	24.93 ± 1.41(4)	0.85 ± 0.03(4)		2.44 ± 0.18(4)
Methylpregnenolone	C	23.50 ± 0.34(4)	1.16 ± 0.07(4)		2.43 ± 0.08(4)
	T	22.67 ± 2.30(3)	1.21 ± 0.13(4)		2.20 ± 0.22(4)
DOCA	C	32.03 ± 2.00(8)	2.10 ± 0.21(5)		1.62 ± 0.10(6)
	T	31.09 ± 1.69(8)	2.24 ± 0.23(5)		1.82 ± 0.15(8)
Acetoxyprogesterone	C	36.75 ± 2.50(4)	2.38 ± 0.11(4)		3.06 ± 0.15(4)
	T	38.38 ± 2.40(4)	2.34 ± 0.10(4)		2.68 ± 0.27(3)

C: control

T: treated

In brackets: number of animals

TABLE III

Effects on the 5 HT amd 5 HIAA content in brain stem

	5-HT			5-HIAA	
	Controls	Treated	P	Controls	Treated
MPA	0.65 ± 0.02 (11)	0.45 ± 0.02 (11)	<0.001	0.45 ± 0.02 (11)	0.43 ± 0.02 (11)
Melengestrol	0.99 ± 0.03 (15)	0.73 ± 0.04 (15)	<0.001	0.39 ± 0.07 (15)	0.40 ± 0.05 (15)
Pregnenolone	0.72 ± 0.02 (9)	0.60 ± 0.01 (9)	<0.0025	0.40 ± 0.03 (9)	0.34 ± 0.02 (9)
α-CH_3-Pregnenolone	0.64 ± 0.07 (4)	0.68 ± 0.09 (3)		0.43 ± 0.05 (4)	0.37 ± 0.07 (4)
17-α-OH-Progesterone	0.58 ± 0.03 (4)	0.52 ± 0.02 (4)		0.50 ± 0.03 (4)	0.42 ± 0.02 (4)
Chlormadinone	0.69 ± 0.02 (8)	0.66 ± 0.02 (8)		0.34 ± 0.01 (8)	0.33 ± 0.01 (8)
DOCA	0.68 ± 0.07 (8)	0.72 ± 0.03 (7)		0.31 ± 0.02 (8)	0.35 ± 0.03 (7)
Acetoxyprogesterone	0.55 ± 0.02 (4)	0.57 ± 0.02 (4)		0.43 ± 0.05 (4)	0.40 ± 0.06 (4)

$\bar{X}$ ± SEM: Results expressed in µg/g fresh tissue

In brackets: number of animals.

TABLE IV

Effect of MPA on different parameters of female rats under 24hs fasting

	L T P (µmol Kyn/g dry t./h)	NEFA (mE/l)	Plasma Trp total (µg/ml)	Plasma Trp free (µg/ml)	Brain stem Trp	5 HT (µg/g f.t.)	5 HIAA
Controls	6.1±0.93 (7)	2.54±0.72 (7)	24.94±1.13 (7)	1.80±0.09 (7)	2.19±0.16 (7)	0.67±0.04 (6)	0.45±0.04 (6)
Treated	9.2±1.02 (7)	1.47±0.20 (6)	25.78±1.11 (7)	1.58±0.09 (7)	2.22±0.09 (7)	0.64±0.03 (6)	0.50±0.06 (6)
P	<0.02	<0.01		<0.01			

Results are expressed in $\bar{X} \pm$ SEM

In brackets: number of animals.

TABLE V

Adrenalectomized rats treated with MPA

Treatment	5-HT		MAO		5-HIAA	
	C	T	C	T	C	T
Adrenalectomized	0.84±0.02	0.82±0.03 (7)	0.13±0.01	0.12±0.01 (7)	0.60±0.07	0.68±0.08 (7)
Sham-operated	0.79±0.02	0.50±0.02 (10)	0.12±0.01	0.13±0.01 (10)	0.38±0.01	0.44±0.05 (10)
		P < 0.001				

$\bar{X}$ ± SEM : results expressed in : MAO : µmol 4-HOQ/g fresh tissue/h.

5-HT, 5-HIAA: µg/g fresh tissue

(C) controls: vehicle.

(T) treated: MPA (8hs).

In brackets: number of animals.

TABLE VI

Rats treated with ACTH and MPA

Treatment	corticosterone	5-HT	MAO	5-HIAA
ACTH + MPA	143.5±7.3 * (4)	0.74±0.05 (4)	0.11±0.01 (4)	0.45±0.02 (4)
ACTH	146.7±28.3 * (4)	0.63±0.10 (4)	0.10±0.01 (4)	0.39±0.03 (4)
Saline	8.75±3.1 (4)	0.63±0.10 (4)	0.09±0.01 (4)	0.35±0.02 (4)

* $P < 0.001$

$\bar{X} \pm$ SEM : results expressed in: µg/g fresh tissue(5-HT, 5-HIAA).
µmol 4-OH Q/g fresh tissue/h. (MAO)
µg/100 ml of plasma (corticosterone)

In brackets: number of animals.

Discussion.

1) Among the steroids herein studied, only MPA and MLA significantly raised LTP activity and both of them lowered the 5-HT content of brain stem (this action could be due to a glucocorticoid effect; Nugent et al., 1975).
Pregnenolone, bioprecursor of steroids, significantly decreased 5-HT but did not modify LTP activity. Most likely, the liver enzyme would act as a regulating mechanism of plasma Trp levels,

(Curzon and Green, 1969), which apparently are not always related to Trp and 5-HT content in the brain. This fact would be confirmed by the results obtained with the concentration of free Trp in rats treated with MPA. Blood sugar levels were not modified by MPA and MLA.

2) In controls under fasting, the NEFA were elevated probably because of the stress caused by fasting, observing a simultaneous increase of free plasma Trp. However, the contents of brain stem Trp, 5-HT and 5-HIAA were not increased.

MPA treatment to fasting rats compared with controls, raised LTP activity, lowered NEFA and free Trp levels, but did not modify brain stem parameters.

3) In adrenalectomized rats, MPA treatment did not modify 5-HT content while it decreased in sham-operated animals, as was expected. This suggests that the high ACTH levels in the blood of adrenalectomized rats, as in those under fasting, antagonizes MPA effect.

4) In order to confirm these findings ACTH and ACTH-MPA (table VI) were injected to another group of animals, observing that exogenous ACTH not only increased plasma corticosterone, but also antagonized the effect of MPA on the 5-HT content of brain stem.

According to Neckers (1975), in adult mice, after stress, the administration of glucocorticoids increased the transport of Trp into brain nerve-endings. The differences with our results in rats, probably are due, not only to the methods employed, but also to the animals used.

5) In all the experiments performed, no modification of the MAO enzymatic system activity was observed.

6) Further studies would be needed to elucidate the mechanism

by which 5-HT diminishes without modifying 5-HIAA. Probably, Tryptophan-hydroxylase has no participation in 5-HT decrease and the antagonism by ACTH exogenous (Kizer et al.,1976). This could be due to a different compartamentalization process (Graham-Smith, 1973; Green et al.,1976) or to a slight increase in the turnover or a slight decrease in synthesis, due to the effect of MPA under our experimental conditions.

Our results do not enable us to determine a relationship between the chemical structure of the steroids studied and the effects observed. This would corroborate the findings obtained by Algeri et al.,(1977) since another progestin compound (Linestrol) increases 5-HT turnover in rat brain.

Our results with certain steroids suggest the participation of 5-HT in the regulation of ACTH secretion; although Rotsztejn et al.,(1977), working in other cerebral areas do not observe that correlation.

The interrelationship between MPA and ACTH in female rats, was demonstrated by Fell et al.,(1967); but in different experimental conditions.

The results would seem to prove the existence of a serotoninergic regulating mechanism of a positive feed-back type. A basal level of ACTH secretion would be modified by normal amounts of 5-HT (probably together with other monoamines). The lack of ACTH liberation would decrease the regulation needs of 5-HT in brain stem (Scapagnini and Preziosi, 1972).

The effect of adrenalectomy on the 5-HT content of the brain is a matter of controversy (Teledgy and Vermes, 1975; cit, by Rotsztejn et al., 1977).

Experiments are in progress in order to obtain more information

about the induction of LTP by corticosteroid hormones and the decrease of 5-HT without modification of MAO activity (Youdim and Holzbauer, 1976), and 5-HIAA contents.

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