with physiological solution and injected in the bath solution in a volume of 0.1 ml every 4 min. Isotonic contractions were registered for 30 sec. After this time the bath solution was changed. Dose effect curves of substance P and of the test substance were registrated on each organ.

Results and discussion. In each experiment the ED_{50}, the maximal contraction and the activity relative to SP (set at 100) were estimated. The ED_{50} SP was $1.9 \pm 0.6 \cdot 10^{-8}$ M, against $5.1 \pm 1.5 \cdot 10^{-8}$ M for the analogue. Thus the relative activity of the analogue was found to be 37±12% and its maximal contraction 98±4%. The identical intrinsic efficacy of [N^5-dimethyl-Gln^6]-SP_{5-11} suggests that alkyl groups on the N^5 of the Gln^6 residue do not interfere with an active element. On the other hand, the carbonyl of the $CONH_2$ portion of the glutaminyl residue in position 5 appears to be an improvable factor for biological response[6], since methylation either causes a detrimental orientation of the active element or inhibits the interaction with the receptor by a steric effect. The observed distinction of the carbonyl groups of the dual Gln^5-Gln^6-moiety in the heptapeptide may allow a further characterization of the particular binding sites and active elements involved in recognition and activation of the specific receptor(s).

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Brain catecholamines and sleep states in offspring of caffeine-treated rats

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Summary. Caffeine was administered in the diet to rats throughout gestation. In the 2 consecutive untreated generations, an increase of parodoxical sleep was observed at maturity. In the 1st generation, the dopamine level was markedly reduced in the locus coeruleus, whereas that of noradrenaline remained constant. The effect was less pronounced in the 2nd generation.

Caffeine, probably the most widely consumed alkaloid, is a central nervous system (CNS) stimulant and acts principally on the brain cortex[1,2]. Kinetic studies have shown that it is very rapidly distributed in the body[3] and via the placenta is transferred to the foetus[4,5]. It also freely passes the blood-brain barrier[6].

Since it has been reported that chlorpromazine[7] and amphetamine[8] induce changes in behaviour[7] and catecholamine metabolism[7,8] in offspring of treated rats, we were interested to investigate possible transmissible effects of caffeine in the CNS. Therefore, caffeine was administered in the diet to rats during their entire gestation. The sleep patterns were analyzed in the offspring of the 1st and 2nd generation. Catecholamines were determined in discrete brain nuclei which it has been suggested play a role in the regulation of sleep[9].

Material and methods. A total of 120 male and female Sprague-Dawley rats (Iffa Credo - St-Germain-sur-Arbresle, France), weighing 235-240 g, were mated 1 male and 2 females per cage. The mating period was terminated after 48 h. The females were then randomly assigned to 4 groups, in which group A was the control and the test groups received diets containing caffeine at 0.0125% for group B, 0.025% for group C and 0.1% for group D admixed in a commercial standard diet (Nafag 850, Gossau, Switzerland). These diets were given ad libitum with tap water during gestation only.

In the 1st generation (F_1), due to the large number of animals, only females were studied. To obtain the 2nd generation (F_2), adult F_1 females of groups A and D were mated with males of group A. Both adult male and female rats of this 2nd generation were studied.

Electrodes were implanted in the cerebral cortex and neck muscles and sleep pattern parameters were measured in 14 rats per group of the 1st generation and 10 rats per group of the 2nd generation when at a weight of 240 g. Recordings of sleep states were performed after an adaptation period of at least 20 days in an airconditioned, sound-proof room (22±1 °C, 50±5% relative humidity). A light-dark cycle of 12 h was maintained, starting at 07.00 h. Waking (W), slow wave sleep (SWS) and paradoxical sleep (PS) were estimated by a double blind visual reading of the cortical electroencephalogram and the electromyogram from the neck muscles over a period of 7 h (from 08.30 to 15.30 h). PS and SWS phases were considered and counted if they lasted more than 10 and 30 sec, respectively.

Each locus coeruleus of 5 animals from each group was excized according to the method of Palkovits[10]. It was then homogenized in 100 µl n-butanol-HCl. The amine derivatives were formed in 20 µl of trifluoroacetic anhydride and acetonitrile (1:1). The derivatives were analyzed by a mass-fragmentographic method, using deuterated analogues as internal standards[11].

Results. The average daily caffeine intake per rat was 0, 2.9±0.1, 5.7±0.1 and 21.5±0.5 mg for groups A, B, C and D, respectively. There were no significant differences in the number of pups per litter, the sex ratio per litter, the mortality or body weight gain of the pups. However, the

Table 1. States of vigilance in offspring of caffeine-treated rats

Group	TS (min)	SWS (min)	PS (min)	Nb. $\overline{SWS}_t$	$\overline{SWS}_t$ (min)	Nb. $\overline{PS}_t$	$\overline{PS}_t$ (min)
First generation							
Females							
A(14)	318.78 ± 10.76	280.80 ± 9.14	37.95 ± 2.81	28.79 ± 1.19	10.05 ± 0.61	18.79 ± 1.05	2.02 ± 0.12
B(14)	328.52 ± 7.77	283.75 ± 6.65	44.71 ± 2.65	31.43 ± 1.49	9.35 ± 0.57	22.79 ± 1.17[a]	1.99 ± 0.11
C(14)	336.50 ± 3.73	278.58 ± 3.39	57.90 ± 2.91[c]	34.79 ± 0.91[c]	8.10 ± 0.28[b]	26.86 ± 0.92[c]	2.16 ± 0.10
D(14)	320.58 ± 8.19	257.00 ± 7.55	63.80 ± 2.29[c]	35.36 ± 1.17[c]	7.40 ± 0.37[c]	27.50 ± 0.89[c]	2.34 ± 0.09[a]
Second generation							
Females							
A(10)	331.61 ± 11.35	287.83 ± 9.02	43.78 ± 2.97	28.50 ± 1.27	10.29 ± 0.44	20.60 ± 1.07	2.12 ± 0.08
D(10)	328.50 ± 5.56	267.89 ± 6.61	60.61 ± 2.42[c]	35.50 ± 1.94[b]	7.83 ± 0.60[b]	28.00 ± 1.05[c]	2.17 ± 0.07
Males							
A(10)	348.19 ± 5.78	299.74 ± 5.09	48.46 ± 1.45	29.90 ± 1.18	10.18 ± 0.44	21.90 ± 0.28	2.22 ± 0.08
D(10)	356.96 ± 4.44	292.84 ± 4.14	64.12 ± 1.55[c]	33.10 ± 0.99	8.73 ± 0.34[a]	26.90 ± 1.07[c]	2.41 ± 0.09

A=0, B=0.0125, C=0.025 and D=0.1% caffeine in the diet of rats during their gestation. Number of animals in brackets. Recording time=7 h; TS=total sleep, SWS=slow wave sleep, PS=paradoxical sleep, Nb. $\overline{SWS}_t$=number of SWS phases, Nb. $\overline{PS}_t$=number of PS phases, $\overline{SWS}_t$=SWS/Nb. $\overline{SWS}_t$=average duration of one SWS phase, $\overline{PS}_t$=PS/Nb. $\overline{PS}_t$=average duration of one PS phase. Values are given ± SEM, Student's t-test: $^a p<0.05$, $^b p<0.01$, $^c p<0.001$.

Table 2. Catecholamine levels in the locus coeruleus of offspring of caffeine-treated rats

Group		1st generation Females group C	Females group D	2nd generation Females group D	Males group D
Dopamine	Control	0.36 ± 0.04	0.40 ± 0.03	0.46 ± 0.04	0.39 ± 0.06
	Treated	0.26 ± 0.03	0.19 ± 0.01	0.34 ± 0.06	0.33 ± 0.06
	% of control	72%	48%	74%	84%
	t-test	$p<0.05$	$p<0.02$	$p<0.1$	NS
Noradrenaline	Control	4.0 ± 0.2	3.6 ± 0.2	4.1 ± 0.2	4.2 ± 0.4
	Treated	4.0 ± 0.3	2.9 ± 0.3	3.6 ± 0.6	4.2 ± 0.8
	% of control	100%	81%	88%	100%
	t-test		NS	NS	

In each series, the results represent the mean of 5 animals (ng/locus ± SEM). Control group=group A. A=0, C=0.025 and D=0.1% caffeine in the diet of rats during their gestation.

food consumption of the rats of groups B and C during the last 2 weeks of gestation was 10% higher than that of groups A and D.

Table 1 includes the detailed sleep pattern for both generations of offspring of the control and the caffeine-treated rats and shows that there was an increase of PS due to an increase in number of PS phases in response to the caffeine treatment. The duration of each phase, however, remained constant. This increase in the number of PS phases was evenly distributed and remained constant over the whole period of recording. Although the average duration of a SWS phase was decreased, the number of phases increased; as a consequence total SWS did not change.

Table 2 shows the catecholamine levels in the locus coeruleus as percentages of the levels in the rats of group A. In the 1st generation, dopamine was markedly decreased whereas noradrenaline remained constant. In the 2nd generation, the decrease of dopamine was less pronounced in the males than the females of group D of the 1st generation and insignificant in the females of group D.

Discussion. The results suggest that oral caffeine administration to rats during gestation provoked dopamine level changes in discrete regions of the brain and a concomitant increase of paradoxical sleep during the period recorded. Since only a part of the circadian rhythm was recorded, an effect on the distribution of the sleep states appearing later cannot be excluded. The decrease of dopamine observed in the locus coeruleus could suggest the existence of dopaminergic neurons in the structure. Blondaux et al.[10], who induced hypersomnia in the cat by isthmic lesions, also observed a decrease of dopamine in the locus coeruleus. Both the neurophysiological and biochemical aspects are currently being further assessed in order to confirm these preliminary results.

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