The effects of dietary vitamin E supplements on reproductive performance of female rats

Dietary vitamin E (IU/kg diet)	No. of animals	Fertility test		Fetus Score[d]		
		Insemination[a] index (%)	Implantation[b] index (%)	Live (%)	Malformed (%)	Resorbed (%)
0	5	80	100	20[e]	0	80[e]
25	5	80	100	91	0	9
2500	5	100	100	90	0	10
10000	5	100	20[e]	100	0	0

[a] Insemination index is 100 × number of rats inseminated/number of rats used for mating. [b] Implantation index is 100 × number of rats with at least one implantation site/number of rats inseminated. [c] Significantly lower than other groups, probability ≤ 0.004 by Freeman and Halton's exact probability test[5]. [d] Fetus score is expressed as 100 × number of live or malformed or resorbed fetuses/number of implantation sites. [e] Significantly different from other groups in the same column, probability ≤ 0.004 by Mann-Whitney U-test[4].

with excess vitamin E supplements were found to be inseminated during the mating period. At necropsy, all inseminated rats fed dietary vitamin E from 0 to 2500 IU/kg diet for 3 months were pregnant. However, only 1 out of the 5 inseminated rats fed 10,000 IU vitamin E/kg diet was pregnant. The low level of fertility in rats fed 10,000 IU vitamin E/kg diet was significantly different from the other 3 groups ($p < 0.004$) using Freeman and Halton's exact probability test[5] for statistical analysis. Fetuses from normal and high vitamin E supplemented rats were otherwise quite normal and no signs of malformations were observed.

In an earlier study, hypervitaminosis E has been reported to disturb ovarian activity in rats[6]. After administration of 3.3 IU vitamin E daily for more than 4 months, it was found that the number of corpora lutea decreased, the weight of ovaries reduced, fewer follicles ripened and the length of estrous cycle altered. The results of our fertility test have demonstrated the possibility of serious reproductive consequences of ovary malfunction induced by hypervitaminosis E. Increased intake of dietary vitamin E has been demonstrated to reduce the serum concentrations of prostaglandins in rats[7]. Since prostaglandins have been shown to play a role in regulating many reproductive processes including ovulation and corpus luteum function[8], the effect of hypervitaminosis E on fertility is quite probably due to its influence on prostaglandins.

Our preliminary results indicate that excess dietary vitamin E, as well as vitamin E deficiency[9], for a prolonged period of time, can interfere with normal reproductive functions in female rats, although the mechanisms may be quite different. Further investigations into the adverse effects of hypervitaminosis E on reproductive functions of female rats are presently being carried out in our laboratory.

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On the mechanism of the amphetamine induced vasodilatation at the rat's cerebral cortex[1]

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Summary. Cerebral cortical blood flow was measured with the hydrogen clearance technique. It was found that the increase in CoBF induced by amphetamine is blocked by atropine or chlorpromazine.

Amphetamine has been reported to increase cerebral blood flow in the rat by a mechanism not dependent on metabolic activation, as the increase in cerebral blood flow is severalfold greater than the change in cerebral metabolic rate of oxygen[3].

The increase in cerebral cortex blood flow (CoBF) associated with electrocortical desynchronization in the urethanized rat has been adscribed to a cholinergic mechanism as it can be blocked by atropine and potentiated by eserine[4]. Also a cholinergic mechanism has been found responsible for the cerebrovascular effect of CO_2 in rats[5]. In order to determine the possible participation of a neurogenic mechanism of the same sort in the action of amphetamine, the present experiments were performed in which drugs affecting synaptic transmission in the central nervous system were assessed on their ability to modify the cerebrovascular effect of amphetamine. As amphetamine is known to release adrenergic transmitters, the effect on CoBF of noradrenaline, adrenaline, isoproterenol and amphetamine itself when topically applied to the cortex was also assessed.

1 This work was supported by grants from Consejo Nacional de Investigaciones Científicas y Tecnicas and Consejo de Investigaciones de la Provincia de Santa Fé, Argentina.
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Table 1. Effect of amphetamine (4 mg/kg) on untreated controls and following pretreatment with atropine (8 mg/kg) or chlorpromazine (10 mg/kg)

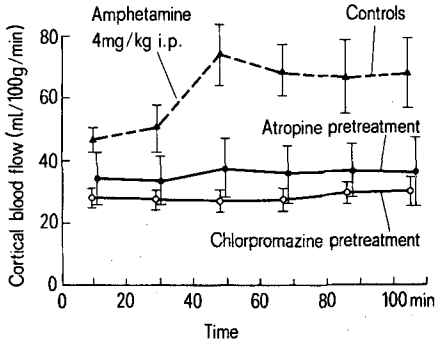
	Co B. flow (ml/100 g min)	Co V. resistance (mm Hg/ml/ 100 g min)	Mean B. pressure (mm Hg)
Untreated controls (mean±SE)	50.18±7.767	1.218±0.204	51±4.78
Amphetamine, 4 mg/kg (mean difference[a]±SE)	23.73±5.143	−0.357±0.121	−2.20±3.74
N	11	5	5
p[b]	<0.001	<0.05	n.s.
Atropine, 8 mg/kg (mean±SE)	33.6±7.584	2.498±0.365	67.87±6.421
Amphetamine, 4 mg/kg (mean difference[a]±SE)	3.6±2.376	0.207±0.152	2.62±3.741
N	10	8	8
p[b]	n.s.	n.s.	n.s.
Chlorpromazine, 10 mg/kg Mean±SE	27.82±3.173	2.145±0.267	53.45±4.747
Amphetamine, 4 mg/kg Mean difference[a]±SE	−0.36±1.591	−0.073±0.117	−2.82±2.607
N	11	11	11
p[b]	n.s.	n.s.	n.s.

[a] Mean ± SE of the difference between the first determination of CoBF after and last before amphetamine injection. [b] Student's t-test for paired samples.

Table 2. Effects of drugs topically applied on cortical blood flow

Drug	CoBF (ml/100 g min) mean ± SE Control[a]	Difference[b]	p[c]	N[d]
Noradrenaline (0.5 mg/ml)	51.9±17.93	−6.58±7.13	n.s.	5
Adrenaline (0.5 mg/ml)	46.6±5.62	3.30±4.72	n.s.	5
Isoproterenol (0.4 mg/ml)	60.29±20.71	12.14±3.96	<0.05	7
Amphetamine (1 mg/ml)	47.87±13.44	−8.47±11.70	n.s.	8

[a] Mean ± SE of CoBF-values previous to drug application. [b] Mean ± SE of the difference between CoBF after and before drug application. A positive value means increase in blood flow and vice versa. [c] Student's t-test for paired samples. [d] Number of animals.



Cortical blood flow before and at successive time intervals after amphetamine injection (4 mg/kg) (arrow) in control animals and after pretreatment with atropine (8 mg/kg) or chlorpromazine (10 mg/kg).

Materials and methods. 57 rats were used. The animals were anesthetized by a single dose (1.5 g/kg) of urethane by the i.p. route. They were tracheostomized and fixed to a nose clamp. The parietal cortex was exposed and a bare platinized platinum electrode, 30 μm in diameter, was inserted within the cerebral cortex by means of a micromanipulator. The electrode tip was placed at 1 mm below cortical surface. Current between the platinum electrode and a calomel half-cell connected by an agar bridge to the s.c. tissue at the neck was recorded in a channel of a Beckman Dynograph recorder. A small flow of hydrogen gas was added to the inspired air and maintained constant until the current from the cortical electrode showed a steady state. Hydrogen flow was then stopped and the desaturation curve was recorded to calculate local blood flow by means of the equation

$$k = \frac{\ln 2}{T^1/_2}$$

where $T^1/_2$ = half time of wash out slope in min. k has the dimensions of ml/ml min. Values were finally expressed as ml/100 g min. One femoral artery was cannulated in order to record the arterial pressure by means of a Statham P23BB pressure transducer connected to a suitable channel of the recorder.

In some experiments, a ring of 3.5 mm internal diameter and 2 mm height was positioned over the cortex by means of a micromanipulator, avoiding pressing the pial vessels. The ring was provided with inlet and outlet tubing connected to syringes and was kept filled with synthetic cerebrospinal fluid that was in direct contact with the pial surface. Drugs were added to the synthetic CSF in order to assess their topical effect on CoBF. The platinum electrode was inserted into the cortex through the center of the ring.

Results. After a single i.p. dose of amphetamine, CoBF significantly increased (table 1 and figure). No blood pressure changes were seen however and cortical vascular resistance also decreased significantly. The increase in CoBF was found at the first determination after injection of the drug and it remained high for the subsequent observation period of 75 min (figure). When the animals were pretreated with atropine or chlorpromazine the effect was completely blocked (figure and table 1). Topical application of amphetamine had no effect on CoBF. Adrenaline and noradrenaline were also without effect but isoproterenol induced about a 20% increase in blood flow (table 2).

Discussion. The original observation by Carlsson et al.[3] reporting an increase in cerebral blood flow measured with the ^{133}Xe method at the saggital sinus in the rat is confirmed by the present experiments. The above mentioned authors ruled out the role of tissue acidosis by measurements of lactate and pyruvate contents and our observations that topical amphetamine is devoid of effect on CoBF confirms that a direct 'metabolic' effect of amphetamine on cerebral cortex is unlikely to be responsible for the changes in CoBF.

Noradrenaline, a transmitter which is present at sympathetic terminals on cortical blood vessels might have been

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released by the action of amphetamine. However, topically applied noradrenaline had no significant effect on local blood flow in the present experimental conditions. Only isoproterenol showed a small effect on CoBF which is in line with previous observations[7,8]. An increasing amount of evidence points to the idea that cerebral blood flow might be under the control of neurogenic mechanisms originating in the brain stem[9-14]. The fact that chlorpromazine[15,16] which is known to depress brain stem mechanisms, completely blocked the cerebrovascular effect of amphetamine suggests that the reported indirect effect of amphetamine on cortical blood vessels might be related to the activation of a dilatatory system originating in the brain stem and projecting to the cerebral cortex. The blockade by atropine of the cerebrovascular effect of amphetamine is suggestive that the proposed brain stem mechanism might include a cholinergic step.

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Are there 2 cholinergic thermoregulatory centres in rats?

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Summary. An attempt was made to replicate the conflicting previous reports of hypo- and hyperthermic effects of intrahypothalamically administered carbachol. Despite using the same coordinates, injection parameters, and strain of rats reported by others, only hypothermia was conclusively demonstrated. It was concluded that the cholinergic system mediates heat loss mechanisms in rats.

There exists a considerable controversy over the role of the cholinergic system in the central control of thermoregulation in rodents. Despite a wealth of studies indicating that acetylcholine (ACh) may mediate heat loss mechanisms[1-6], some investigators still maintain that ACh is involved in heat gain mechanisms and have even developed elegant models of thermoregulation on the basis of scanty evidence[7-10]. Since there were some variations in technique and other procedures between these conflicting studies, it was decided to attempt to replicate two of the key experiments in this area[11,12]. The results of the present studies are consistent with the view, expressed by the majority of research workers in this field, that a central cholinergic system mediates heat loss mechanisms in rats.

In one experiment female Sprague-Dawley rats, approximately 130–190 g and 70 days old, were unilaterally

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Maximum deviation of temperature and time to reach maximum effect after intrahypothalamic injections of carbachol

Treatment group	N	Sex	Coordinates	Maximum deviation (°C) (mean ± SEM)	Time for response (min) (mean ± SEM)
2 µg carbachol / 1 µl saline	6	F	AP 1.8 ML 1.5 DV − 8.5	− 2.7 ± 0.3	27 ± 1
2 µg carbachol / 1 µl saline	3	M	AP 1.7 ML 0.8 DV − 8.5	− 1.1 ± 0.4	20 ± 4
5 µg carbachol / 0.5 µl saline	6	M	AP 1.7 ML 0.8 DV − 8.5	− 2.1 ± 0.5	34 ± 8