Superior avoidance learning in the offspring of rats treated with an anabolic steroid during pregnancy

A. Bertolini, M. Bernardi, R. Poggioli, S. Genedani, M. Castelli and W. Ferrari

Institute of Pharmacology, University of Modena, via G. Campi 287, I-41100 Modena (Italy), 28 July 1978

Summary. Treatment of pregnant rats with stanozolol, an anabolic steroid, increases the avoidance learning of the offspring at maturity.

In mammals, brain development is maximal during pregnancy (except for cerebellum) and the period in which cortical neurons are capable of dividing is relatively very short: in mice and rats brain development ceases around birth, whereas in man it ceases long before birth, at about the end of the 5th month of pregnancy[1,2]. It is therefore well conceivable that stimulation or inhibition of mitoses during pregnancy will be most effective for cells, like neurons, with a short and well-defined proliferation period. Severe retardation in brain growth of the pups of dams undernourished during the period of gestation has in fact been observed and such retardation is irreversible, even if adequate feeding is provided during the lactation period[3].

On the other hand, offspring of rats maintained on a normal diet and treated with growth hormone during pregnancy, have larger brains (mainly due to the increased number of cortical neurons) and, at maturity, demonstrate superior learning ability[4-8]. Since growth hormone does not cross the placenta[9], it has been proposed that its action must be mediated by a second messenger, such as somatomedin, or a similar trophic substance, able to directly influence fetal brain growth[8].

In order to ascertain whether such a property is shared also by other and more accessible proteo-anabolic substances, we have studied the effect of stanozolol[10] (an anabolic steroid with a very favourable myotrophic: androgenic dissociation index[11]) administered to pregnant rats, on the avoidance learning of the offspring at maturity.

Materials and methods. Pregnant rats of a Wistar strain (Morini, S.Polo d'Enza, Reggio Emilia, Italy), with free access to a standard diet (MIL-rats, Morini) were used. They weighed 220-250 g prior to mating and the time of conception was determined by vaginal smears taken each morning.

When pregnant, the rats were randomly divided into 2 groups of 10: experimental animals received daily s.c. injections of 0.5 mg/kg stanozolol (17-methyl-5α-androstan-{3,2-c}pyrazol-17β-ol) dissolved in corn oil, from days 7 to 21 of gestation; control animals received the same volume of corn oil.

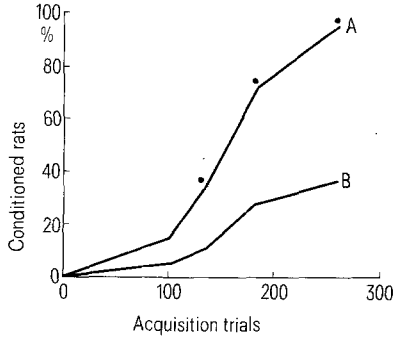
At birth, half the offspring of each rat was killed by decapitation and cerebral hemispheres were weighed; the remaining animals, when 60 days old (150-170 g), were used for the avoidance learning study. Avoidance conditioning was carried out in a shuttle box, divided into 2 equal compartments with a grid floor through which the unconditioned stimulus of shock (25 V, 1.8 mA) could be delivered to the feet of the rats. The conditioned stimulus was the sound of a buzzer which lasted for 5 sec before the unconditioned stimulus was given. The conditioned stimulus terminated as soon as the rat had crossed the barrier between the compartments; when the response occurred within 5 sec, the rat avoided shock. 15 trials were carried out each day with 40-sec intervals between them and the criterion of learning was satisfied when 12 or more avoidances per session, during 3 consecutive sessions, occurred.

Rats which satisfied the above criterion were studied for the rate of extinction. The schedule of the extinction trials was the same as that used during acquisition, except that only the conditioned stimulus was given: if rats had not crossed the barrier within 5 sec, the conditioned stimulus was terminated. The extinction period started immediately after the learning period and lasted for 14 days.

Results and discussion. At the dose used, stanozolol had no effect on pregnancy, the mean number and weight of fetuses and the number of resorptions were quite similar, and no gross malformation was observed. The percentage weight of the cerebral hemispheres was slightly, but not significantly, greater in the offspring of the stanozolol-treated rats (2.461 ± 0.05 vs 2.398 ± 0.07).

On the other hand, avoidance conditioning was far quicker in the offspring of the treated rats and at the end of the acquisition period 95% of them had satisfied the criterion of learning, versus the 38% of the offspring of control rats (figure). The rate of extinction was similar in the 2 groups.

From these preliminary data, it would appear that stimulating protein anabolism with a nontoxic dose of anabolic steroids during the proliferation period of neurons results in an increased avoidance learning at maturity. Further experiments are underway in order to clarify whether the number of cortical neurons is increased, whether this is a property shared by other anabolic steroids and whether the reproductive function of these rats is not compromised by such a treatment during the fetal period.



Rate of avoidance conditioning in the offspring of rats treated with stanozolol (0.5 mg/kg, s.c.) from days 7 to 21 of gestation (A) and in the offspring of controls (B). ● $p < 0.01$ (χ-square-test, corrected according to Yates).

1 J.B. Angevine and R.L. Sidman, Nature *192*, 766 (1961).
2 M. Berry, A.W. Rogers and J.T. Eayrs, Nature *203*, 591 (1964).
3 T. Goswami and U. Srivastava, Can. J. Physiol. Pharmac. *56*, 274 (1978).
4 B.G. Clendinnen and J.T. Eayrs, J. Endocr. *22*, 183 (1961).
5 J.B. Block and W.B. Essman, Nature *205*, 1136 (1965).
6 S. Zamenhof, J. Mosley and E. Schuller, Science *152*, 1396 (1966).
7 S. Zamenhof, E. van Marthens and L. Granel, Science *174*, 954 (1971).
8 V.R. Sara, L. Lazarus, M.C. Stuart and T. King, Science *186*, 446 (1974).
9 Z. Laron, S. Mannheimer and S. Guttman, Experientia *22*, 831 (1966).
10 R.O. Clinton, A.J. Manson, F.W. Stanner, A.L. Beyler, G.O. Potts and A. Arnold, J. Am. chem. Soc. *81*, 1513 (1959).
11 G.O. Potts, A. Arnold and A.L. Beyler, Handbk exp. Pharmac. *43*, 383 (1976).