

## **Effect of long-term treatment with high doses of guanethidine on sperm transport and fertility of rats**

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### **Summary**

1. Male rats treated with guanethidine 25 mg/kg daily for eight weeks had no demonstrable ejaculatory function immediately after cessation of treatment. Four weeks later, ejaculatory function had returned in two-thirds of the males, and after eight weeks, all males were able to deposit sperm in the vagina. However, fertility had returned at that time in only two of the nine males. On the basis of these findings it might be expected that fertility would return in the other males after a more prolonged observation period.
2. At autopsy, 66 days after cessation of treatment, the vasa deferentia were congested and filled with viscous material, and along their course dilated regions and pseudocysts containing sperm debris and spermatozoa were found.
3. The isolated ducts of the guanethidine-treated males showed a markedly increased response to noradrenaline if compared with ducts from control rats. Response to tyramine was similar in both treated and untreated rats. In view of the increased response to noradrenaline it may be concluded that the noradrenaline stores, which are sensitive to tyramine, were still reduced 66 days after guanethidine treatment had been stopped.

### **Introduction**

Prolonged parenteral administration of guanethidine to rats may damage peripheral sympathetic neurones, the severity and reversibility of the lesions depending on the doses and on the duration of treatment. In the superior cervical ganglion of rats the number of cells was markedly reduced, and abnormal cell vacuolation and severe changes in mitochondrial structure were found (Burnstock, Evans, Gannon, Heath & James, 1971; Jensen-Holm & Juul, 1971). Fluorescence of adrenergic nerve fibres disappeared in most tissues that were studied (Burnstock *et al.*, 1971). In the vas deferens and the genital glands, neuronal catecholamine stores were depleted (Gannon, Iwayama, Burnstock, Gerkens & Mashford, 1971). After cessation of treatment, fluorescence reappeared only slowly and after six months had not reached the intensity seen before guanethidine had been given. Although inhibition of the ejaculation reflex by guanethidine had been reported shortly after the drug became available for antihypertensive treatment, little is known of its effects on fertility. In the present study we attempted to determine the influence that impaired sympathetic innervation of male sex organs has on the fertility of male rats, immediately after, and at intervals of four and eight weeks after intramuscular administration of high doses of guanethidine daily for eight weeks.

## Methods

Ten male Sprague-Dawley rats weighing between 235 and 295 g received guanethidine sulphate (Ismelin, CIBA) at a dose of 25 mg/kg daily for a period of eight weeks. The drug was administered in a 1% solution and injected into the hindleg muscles. Ten control rats weighing between 235 and 270 g received daily injections of similar volumes of 0.9% w/v NaCl solution (saline). All animals were kept in individual cages and were weighed every three days. Shortly after the last injection, blood pressure was measured in all animals by means of a piezoelectric method (W + W Electronic, Basel).

One day after the last injection, each male was mated with two females. Vaginal smears had previously been taken from the females to obtain information on their oestrous cycles. Males and females were left together for ten days, and every morning between 8 and 9 a.m. a vaginal smear was taken from all the females and stained with Löffler's methylene blue. As soon as sperms were found in the smear, the female was taken away from the male. The same procedure was repeated four and eight weeks after guanethidine treatment had been stopped, the males being mated with two other females after each interval.

After the third period of pairing, i.e. 66 days after treatment with guanethidine had been stopped, the male rats were killed and the vasa deferentia prepared. Pieces of the ducts 2.5–3 cm in length were placed in an organ bath of modified Krebs-Henseleit solution bubbled with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 37° C. Preload was 1.7 g, and isometric contractions were recorded by means of a Sanborn microforce transducer FTA 10-1 and a servo chart recorder. Noradrenaline ((-)-noradrenaline hydrochloride, Arterenol, Hoechst A.G., Frankfurt a.M.) was added to the bath in concentrations of 10<sup>-7</sup> to 5 × 10<sup>-6</sup>M, and tyramine (tyramine chloride, E. Merck, Darmstadt) in concentrations of 10<sup>-5</sup> and 5 × 10<sup>-5</sup>M.

## Results

In general, the daily injections of guanethidine were well tolerated. The gain in weight of treated rats was similar to that of the controls, which received daily injections of saline, and no significant difference in weight between the groups was observed at any time during the experiment. In the treated rats, motor activity was reduced, and a marked ptosis was observed. In the eighth week, one rat died after severe convulsions. At autopsy a large cerebral abscess was found, which probably had no relation to the guanethidine treatment. Mean blood pressure of the animals at the end of treatment was slightly lower (103 ± 5.3 S.E. mmHg) than that of the controls (115 ± 5.37 S.E. mmHg), the difference not being of statistical significance.

**Fertility** During the first ten-day mating period, immediately after cessation of treatment, no sperms were found in the vagina of any of the 18 female rats. No copulation was noted, but the rats were not continuously observed. In 16 females, prolonged dioestrus, up to two weeks, occurred, implying pseudopregnancy. Oestrus became normal within four days after the males had been removed. In all 20 females mated with the 10 control males, sperms were found in the vaginal smears, and after 21 days 19 had litters of between 6 and 17 young (Table 1).

TABLE 1. *Vaginal smears of female rats mated with control rats during ten days*

Rat No.	Days										Size of litter
	1	2	3	4	5	6	7	8	9	10	
1a	—	—	—	—	—	—	—	+			17
1b	+										13
2a	—	—	—	—	—	—	—	+			13
2b	+										13
3a	—	—	+								—
3b	—	+									14
4a	—	—	—	+							6
4b	+										12
5a	—	+									15
5b	—	—	—	—	—	—		+			12
6a	—	—	—	—	—	—	+				17
6b	—	+									11
7a	—	—	—	—	—	—	—	+			14
7b	—	—	—	—	—	—	+				17
8a	—	+									13
8b	+										12
9a	—	+									17
9b	—	+									14
10a	—	—	+								15
10b	—	—	—	—	—	—	—	—	—	+	13

Each control rat paired with two females. +, Sperms found in the vaginal smear.

TABLE 2. *Vaginal smears of female rats mated with guanethidine-treated rats four weeks after cessation of treatment*

Rat No.	Days										Size of litter
	1	2	3	4	5	6	7	8	9	10	
11a	—	—	—	—	—	—	—	—	+		—
11b	—	—	—	+							—
12a	—	—	—	—	—	—	—	—	—	—	—
12b	—	—	—	—	—	—	—	—	—	—	—
13a	—	—	—	—	—	—	—	—	—	—	—
13b	—	—	—	—	—	—	—	—	—	—	—
14a	—	—	+								5
14b	—	—	—	—	—	—	—	—	—	—	—
15a	—	—	+								—
15b	—	—	—	—	+						—
16a	—	—	—	—	—	—	—	—	—	—	—
16b	—	—	—	—	—	—	—	—	—	—	—
17a	+										—
17b	—	—	—	—	—	—	—	—	—	—	—
18a	+										—
18b	—	—	—	—	—	—	—	—	—	—	—
19a	—	—	+								—
19b	+										—

+, Sperms found in the vaginal smear.

During the second period of mating, four weeks after cessation of guanethidine treatment, sperms, originating from six males, were found in the smears of nine females, but only one of them had a litter of five young (Table 2).

When eight weeks had passed after the treatment, the animals were paired again. In 17 of the 18 females, sperms were detected in the smears. Only three of the 17 females had litters of 13, 11, and 10 young respectively, two of these females having been mated with the same male. Hence, in two of the nine males treated, fertility had returned (Table 3).

TABLE 3. Vaginal smears of female rats mated with guanethidine-treated rats eight weeks after cessation of treatment

Rat No.	Days										Size of litter
	1	2	3	4	5	6	7	8	9	10	
11a	—	—	—	+							13
11b	—	+									10
12a	—	+									—
12b	—	—	+								—
13a	—	—	—	+							—
13b	—	+									—
14a	—	+									—
14b	—	—	+								—
15a	—	+									—
15b	—	—	—	—	—	—	—	—	—	—	—
16a	—	—	—	—	+						—
16b	—	+									11
17a	—	+									—
17b	—	—	+								—
18a	—	—	—	+							—
18b	+										—
19a	—	—	+								—
19b	—	—	+								—

+, Sperms found in the vaginal smear.

### Morphology and function of the deferent ducts

At autopsy the vasa deferentia were about twice the diameter of those of the controls and were filled with a white viscous fluid. Along the two ducts, several cystic distensions of the size of a lentil or small pea and of yellowish colour were seen. The cysts were filled with sperm detritus and inspissated sperm, whereas the fluid in the deferent ducts contained large amounts of spermatozoa.

The isolated ducts had a markedly increased sensitivity to noradrenaline compared with ducts taken from control rats. The contraction force developed was

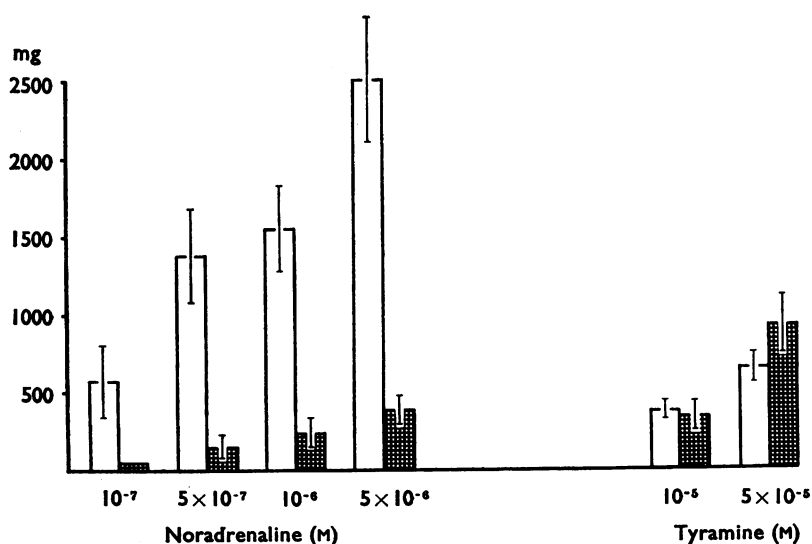


FIG. 1. Response of isolated vasa deferentia to noradrenaline and to tyramine eight weeks after cessation of guanethidine treatment. Isometric contractions. Ordinates: contraction force in mg. Open columns: preparations from rats that had received guanethidine; hatched columns: preparations from control rats.

about six times that of the control organs (Fig. 1). The response of the duct preparations to tyramine did not differ in treated and control rats. If comparable amounts of noradrenaline were released from ducts of both treated and control males, a more marked contraction should have resulted in the highly sensitive ducts of treated rats. Since the responses in the two groups were similar, it may be assumed that tyramine released much less noradrenaline from the ducts of treated rats than from those of untreated controls. When the ratio between responses to tyramine and to noradrenaline was calculated, a markedly diminished response to tyramine was evident for the ducts from guanethidine-treated rats (Table 4).

TABLE 4. *Ratio of tyramine response to noradrenaline response of vasa deferentia preparations of rats treated previously with guanethidine and of control rats*

Rats treated with guanethidine		Controls	
Tyramine $1 \times 10^{-5}$ M	$=0.27$	Tyramine $1 \times 10^{-5}$ M	$=2.26$
Noradrenaline $5 \times 10^{-7}$ M		Noradrenaline $5 \times 10^{-7}$ M	
Tyramine $5 \times 10^{-5}$ M	$=0.25$	Tyramine $5 \times 10^{-5}$ M	$=3.95$
Noradrenaline $1 \times 10^{-6}$ M		Noradrenaline $1 \times 10^{-6}$ M	

## Discussion

Long-term intramuscular administration of high doses of guanethidine resulted in a severe functional impairment of sperm transport, which, within a period of eight weeks after the end of the treatment, was only partly restored. After an interval of only four weeks, ejaculation was possible in some of the treated animals, but fertility had returned in only one of nine males. After eight weeks, sperms were found in the smears of most females, but only two males were fertile. Since the males were separated from the females as soon as the first sperms were found in the vaginal smears, the possibility cannot be excluded that, after several days of pairing, successful impregnation might have occurred in a larger number of females. It may be that under the experimental conditions chosen, damaged sperms, which had been in the swollen ducts for some time, were the first to be deposited in the vagina, and in further copulations more viable sperms might have been ejaculated. The spermiostasis observed had not only led to marked congestion of the ducts, but also to pseudocysts that had been formed by distension of the wall. The pseudocysts were filled with a thick, very viscous material, full of sperm fragments, but also some normal looking sperms.

The increased sensitivity of the vasa deferentia to noradrenaline is in agreement with observations reported by others that guanethidine induces a marked depletion of fluorescent nerve fibres both in newborn and adult rats (Burnstock *et al.*, 1971; Eränkö & Eränkö, 1971). The diminished response to tyramine even two months after cessation of guanethidine-treatment suggests that the tyramine-sensitive catecholamine pools are still depleted. Despite the reduction of the tyramine-induced noradrenaline release and the high sensitivity to noradrenaline, which corresponds to an organ devoid of, or with reduced adrenergic innervation, the ability to ejaculate was restored in all nine rats. However, only two of the males were fertile. It cannot be decided whether, in the remaining seven males, fertility would have been

demonstrable if the animals had not been separated after sperm had been found in the vaginal smear. The fact that two of the males produced litters of normal size makes it probable that in the remaining seven animals a similar functional restoration might also have been expected after a longer time had elapsed. Since it is known that the reduction in fluorescence of nerve fibres is still demonstrable four months after treatment with guanethidine (Burnstock *et al.*, 1971), it may be concluded that not only ejaculatory function, but also fertility can be restored, at least in part, despite the persistence of morphological changes.

## REFERENCES

- BURNSTOCK, G., EVANS, B., GANNON, B. J., HEATH, J. W. & JAMES, V. (1971). A new method of destroying adrenergic nerves in adult animals using guanethidine. *Br. J. Pharmac.*, **43**, 295-301.
- ERÄNKÖ, O. & ERÄNKÖ, L. (1971). Histochemical evidence of chemical sympathectomy by guanethidine in newborn rats. *Histochem. J.*, **3**, 451-456.
- GANNON, B. J., IWAYAMA, T., BURNSTOCK, G., GERKENS, J. & MASHFORD, M. L. (1971). Prolonged effects of chronic guanethidine treatment on the sympathetic innervation of the genitalia of male rats. *Med. J. Aust.*, **2**, 207-208.
- JENSEN-HOLM, J. & JUUL, P. (1971). Ultrastructural changes in the rat superior cervical ganglion following prolonged guanethidine administration. *Acta pharmac. tox.*, **30**, 308-320.

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