J. Pharm. Pharmac., 1970, **22**, 129

LETTERS TO THE EDITOR

Influence of pretreatment with phenobarbitone on the ultrastructure of adrenergic nerve endings in guinea-pig seminal vesicles

Neither angiotensin nor tyramine contract the guinea-pig isolated seminal vesicle (Gascon & Walaszek, 1968; Gascon & Vaillancourt, 1969). After the addition of adrenaline, however, both compounds induce a contraction which exhibits tachyphylaxis. Furthermore, it has been shown (Gascon, Brodeur & Vaillancourt, 1969; Gascon & Brodeur, 1969) that chronic pretreatment of the guinea-pig with testosterone, phenobarbitone or other microsomal enzyme inducers, causes a marked increase in the reactivity of the isolated seminal vesicles to angiotensin and to tyramine.

We now report the influence of sub-acute pretreatment with phenobarbitone on the ultrastructure of the adrenergic nerve endings located in the guinea-pig seminal vesicle.

Twenty male guinea-pigs, 200–225 g, were divided at random into two groups. The animals of the first group were injected intraperitoneally with phenobarbitone (50 mg/kg daily) for 4 days, while those of the second group were given saline. 24 h after the last injection, the seminal vesicles were removed and immersed in a fixative mixture of glutaraldehyde-acrolein at 4°.

The middle portion of the organ was diced into blocks measuring a few millimetres; the blocks were kept for 1h in the fixative, and then post-fixed in osmium tetroxide for 3h (Caulffeld, 1957), dehydrated in graded ethanols, and embedded in Epon 812 (Luft, 1961). Thin and ultrathin sections were cut on a Porter–Blum ultramicrotome, and triple-stained with lead, uranyl acetate, and lead again, for 5, 1 and 8 min, respectively (Sandborn, 1966). The ultrathin sections were examined in a Hitachi electron microscope HS–7S. The population of the small dense-core vesicles (40–60 nm), representing the neuro-transmitter noradrenaline (Wolfe, Potter & others, 1962; Bloom & Barrnett, 1966), was counted on 240 electron micrographs of nerve endings selected at random. In this experiment, 720 nerve endings were used, to ensure a total count of at least 3000 small dense-core vesicles.

There were slightly more than twice as many dense-core vesicles in the phenobarbitone pretreated than in the control nerve endings. The statistical analysis made on the absolute numbers of noradrenaline vesicles showed a highly significant difference ($P<0{\cdot}001$) between the controls and the pretreated animals.

It has been reported (Gascon & Walaszek, 1968; Gascon & Vaillancourt, 1969) that angiotensin and tyramine induce a contraction of the guinea-pig isolated seminal vesicle by releasing endogenous catecholamine. Furthermore, it has been shown (Gascon & others, 1969; Gascon & Brodeur, 1969) that drugs such as testosterone and phenobarbitone, two microsomal enzyme inducers (Conney, 1967), modify the reactivity of the seminal vesicles to both agonists. This effect was attributed by Gascon and his co-workers to an enhanced synthesis of endogenous catecholamine. The increase in the population of the small dense-core vesicles reported here constitutes a strong argument in favour of this assumption. To our mind, this effect of phenobarbitone can be explained by an increase in the synthesis of new noradrenaline vesicles in the nerve endings located in the guinea-pig seminal vesicles. This hypothesis is supported by the studies of Mueller & Shideman (1968), who reported a phenobarbitone-induced decrease in the replenishing time of noradrenaline in the

hearts of animals previously treated with reserpine, and by the unpublished observations of Gascon & Cloutier, which showed a significant increase in the noradrenaline content of the seminal vesicles in animals so treated.

This work was supported by the Medical Research Council of Canada; Grants: MA–3084 and MA–3130.

Département de Pharmacologie,
Faculté de Médecine,
Université de Montréal,
Montréal, P.Q., Canada.

M. G. CÔTÉ
A. BLOUIN
ANDRÉ GASCON

September 16, 1969

REFERENCES

BLOOM, F. E. & BARRNETT, R. J. (1966). *Nature, Lond.*, **210**, 599–601.
CAULFIELD, J. D. (1957). *J. biophys. biochem. Cytol.*, **3**, 827–829.
CONNEY, A. H. (1967). *Pharmac. Rev.*, **19**, 317–366.
GASCON, A. L. & BRODEUR, J. (1969). *Can. J. Physiol. Pharmac.*, **47**, 947–952.
GASCON, A. L., BRODEUR, J. & VAILLANCOURT, M. (1969). *J. Pharm. Pharmac.*, **21**, 325–326.
GASCON, A. L. & VAILLANCOURT, M. (1969). *Archs int. Pharmacodyn. Thér.*, **180**, 134–142.
GASCON, A. L. & WALASZEK, E. J. (1968). *Ibid.*, **175**, 265–272.
LUFT, J. H. (1961). *J. biophys. biochem. Cytol.*, **9**, 409–414.
MUELLER, R. A. & SHIDEMAN, F. E. (1968). *Biochem. Pharmac.*, **17**, 451–467.
SANDBORN, E. B. (1966). *Can. J. Physiol. Pharmac.*, **44**, 329–338.
WOLFE, D. E., POTTER, L. T., RICHARDSON, K. C. & AXELROD, J. (1962). *Science, N.Y.*, **138**, 440–442.

Ulcerative colitis in rabbits fed degraded carrageenan

We recently described the occurrence of ulcerative colitis in guinea-pigs fed orally in their drinking water degraded and undegraded carrageenan derived from the red seaweed *Eucheuma spinosum* (Watt & Marcus, 1969). The lesions produced by degraded carrageenan were severe and involved the whole length of the colon.

We have found that degraded carrageenan derived from the same seaweed also causes severe damage to the colon of the rabbit. In this communication we report on the incidence of ulcerative lesions found in rabbits fed various concentrations of the degraded product.

Twenty male Californian rabbits of 2950 g average body weight were housed in separate cages and fed a standard cube diet (S.G.1). Three experimental groups, 5 rabbits in each group, received as drinking fluid 5, 1 and 0·1% respectively aqueous solutions of degraded carrageenan derived from *Eucheuma spinosum.** The solutions of degraded carrageenan were freshly prepared daily, stored at 4°, and supplied *ad lib* in drinking bottles which were cleaned each day. The volume of fluid consumed per animal per day was measured throughout the 6 to 12 week period of the experiment. Control animals received water *ad lib* but without added carrageenan. At weekly intervals, the animals were weighed and their faeces examined for occult blood using the Haematest method. At the end of the experiment, the animals were killed with pentobarbitone. At post-mortem examination, the colon was removed, emptied of faeces and examined for the presence of ulcerative lesions.

Animals fed degraded carrageenan at the 5% concentration in their drinking water received on average a daily dose of 1·4 g/kg weight over a 6 week period. Diarrhoea associated with visible and occult blood in the faeces developed by the end of 7 days and persisted. The animals rapidly lost weight, the average loss at the end of the