

Cholinesterase activity in the plasma and acid-secretory gastric mucosa of normal, castrated, and castrated oestrogen-treated male rats

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Cholinesterase activity in plasma and fundic mucosa of male, normal, castrated, and castrated and oestrogen-treated rats has been measured. Castration significantly elevated cholinesterase activity in both plasma ($0.02 < P < 0.05$) and fundic mucosa ($P < 0.001$). Castrated rats given 17β -oestradiol ($120 \mu\text{g}$, s.c. daily for 10 days) had enzyme activity in plasma and fundus not significantly different from that in normal rats.

Oestrogen treatment decreases the stimulant effects of histamine, gastrin or carbachol on gastric acid secretion in the male or female rat (Amure & Omole, 1970; Amure, Ginsburg & Omole, 1970). Sawyer & Everett (1946) observed that serum cholinesterase concentrations in rats were increased in conditions where sustained elevation of oestrogen concentrations exist and Everett & Sawyer (1946) reported that serum cholinesterase immediately declined in ovariectomized rats and that oestrogen administration to castrated rats of either sex raised serum cholinesterase levels. Elevated cholinesterase concentration in the plasma, or in the fundic mucosa, where parietal cells reside, may be implicated in oestrogen-induced reduction in rat stomach sensitivity as accelerated destruction of acetylcholine released from vagal nerve endings at parietal cell sites would diminish the booster effect of vagal innervation on sensitivity of parietal cells to secretagogues (Uvnas, 1942). In this work, I have estimated the effects of castration and oestrogen treatment on cholinesterase enzyme concentrations in the male rat.

METHODS

Cholinesterase activity was estimated in 10 normal adult male Wistar rats, 12 castrated male rats (7-10 days post gonadectomy) and 12 castrated male rats given 17β -oestradiol ($120 \mu\text{g}$, s.c.) daily for 10 days.

Collection of plasma and fundic mucosa

A carotid artery of animals under light ether anaesthesia was exposed, cannulated and blood collected into a heparinized plastic tube with thorough mixing. The blood samples were centrifuged at 3000 rev/min for 50 min at 31° and 1 ml of clear plasma recovered from each rat. The rats were then bled to death via the carotid cannula and their stomachs excised, the pyloric region removed and the fundus cleaned with distilled water before scraping the mucosa from each specimen.

Estimation procedure

Cholinesterase was estimated by a modification of the manometric procedure of Augustinsson (1957) using a Gilson Differential Respirometer. The bicarbonate

buffer solution, pH 7.4, used for enzyme determination was prepared by mixing 100.0 ml of 0.15M NaCl, 30.0 ml of 1.26% NaHCO₃ and 2.0 ml of 1.76% MgCl₂ 6H₂O. A 3% w/v solution of acetylcholine iodide powder (BDH) in alkaline buffer was used as substrate for the estimation of total cholinesterase activity (specific + non-specific enzyme). For each estimation, either rat fundic mucosa, 90–200 mg pooled from 2–3 rats homogenized in 5.6 ml of bicarbonate buffer, or 0.5 ml of plasma, mixed with 5.1 ml of bicarbonate buffer, was placed in the main compartment of a Warburg flask while 0.40 ml of the substrate solution was put in the side tube. Control samples for the detection of any spontaneous generation of carbon-dioxide were made in four flasks, two containing 5.6 ml buffer in the main compartment and 0.4 ml of substrate in the side tube, the third containing 100 mg fundic mucosa and 5.6 ml buffer both in the main compartment, the fourth containing 0.5 ml plasma and 5.1 ml buffer also both in the main compartment. Student's *t*-test was used to assess the significance of differences between means and values of $P \leq 0.05$ were regarded as statistically significant.

RESULTS

Cholinesterase activity in the plasma and fundic mucosa of the three groups of rats is shown in Tables 1 and 2.

Table 1. *Total cholinesterase activity in rat plasma.*

$\mu\text{l CO}_2/\text{ml plasma in 30 min at N.T.P.}$			
	Normal male rats (5 rats)	Castrated male rats (5 rats)	Castrated β -oestradiol treated male rats (5 rats)
	298.9	272.5	200.8
	415.1	454.1	220.5
	318.7	449.1	318.5
	224.1	455.7	259.6
	238.9	463.3	252.3
Mean	298.1	418.9	250.3
\pm s.e.	33.9	36.6	20.1

Table 2. *Total cholinesterase activity in rat fundic mucosa.* (Mucosa from 2 to 3 rats were pooled up for each determination).

$\mu\text{l CO}_2/\text{mg fundic mucosa in 30 min at N.T.P.}$			
	Normal male rats (10 rats)	Castrated male rats (12 rats)	Castrated β -oestradiol treated male rats (12 rats)
	0.81	1.75	0.79
	0.95	1.57	0.85
	1.28	1.56	1.12
	1.52	1.63	0.97
	0.60		1.12
Mean	1.03	1.63	0.97
\pm s.e.	0.16	0.04	0.07

Normal male rats

The enzyme activity of normal rats did not vary significantly either in the plasma or the fundic mucosa. The activity was found to be $298.1 \pm 33.9 \mu\text{l CO}_2/\text{ml}$ in

30 min at N.T.P. (mean \pm s.e. in 5 estimations) for the plasma and $1.03 \pm 0.16 \mu\text{CO}_2/\text{mg}$ in 30 min at N.T.P. (mean \pm s.e. in 5 estimations) for the mucosa.

Castrated rats

Castration significantly elevated cholinesterase activity in both the plasma ($0.02 < P < 0.05$) and fundic mucosa ($P < 0.001$). When the castrated animals were given 17β -oestradiol injections, enzyme concentrations were reduced to the values in the normal male rats. Enzyme activity between the normal and castrated oestradiol treated rats in either the plasma ($0.2 < P < 0.3$) or the mucosa ($P > 0.9$) was not statistically different.

DISCUSSION

Castration of male rats produces inhibition of acid gastric secretion (Amure & Omole, 1970) which could be attributed to the elevation of cholinesterase activity. However, oestrogen treatment of castrated rats in doses that had previously been shown to inhibit acid gastric secretion (Amure & Omole, 1970) failed to elevate further the cholinesterase activity in both the mucosa and plasma. Thus, elevation of cholinesterase activity after oestrogen treatment in the castrated male rat (Everett & Sawyer, 1946) could not be confirmed.

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