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The effects of 17β-oestradiol or testosterone on the response to *S*-warfarin in castrated male rats

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The effects of castration alone, or followed by administration of 17β-oestradiol or testosterone, on the action of *S*-warfarin have been investigated in male Wistar rats castrated at 4 weeks of age. Six to 10 weeks later, blood samples were taken before, and at 24 h and 7 days after onset of administration of either 17β-oestradiol (50 to 500 μg kg^{-1} day^{-1} i.p.), testosterone (500 μg kg^{-1} day^{-1} i.p.), or vehicle (polyethylene glycol 200 i.p.). On the seventh day of these interventions, *S*-warfarin (6·3 mg kg^{-1} i.p.) was given; prothrombin complex activity (PCA) was then measured over the next 72 h. In addition, a group of sham-operated rats were given *S*-warfarin, alone. The control prothrombin times were lower in castrated rats (17·9 ± 0·4 s) than in sham-operated rats (19·1 ± 0·6 s). Warfarin, over the first 24 h of its administration, caused a significant ($P \leq 0.01$) fall in PCA in each group of rats, the fall in PCA in castrated rats pretreated with vehicle being significantly ($P \leq 0.05$) less than that seen in the sham-operated rats. The fall was the same in castrated rats given vehicle or those given 17β-oestradiol. In contrast, warfarin caused a greater fall in PCA in the presence of testosterone than in the other groups of castrated rats.

It was found recently that male rats and mice were more sensitive to the coumarin anticoagulant, difenacoum, than female rats and mice (Winn et al 1987). This is consistent with findings that male rats are appreciably more sensitive than female rats to the anticoagulant effects of dietary vitamin K deficiency (Johnson et al 1960; Matschiner & Bell 1973). Interestingly, the administration of oestradiol appeared to delay the onset of anticoagulation produced by dietary vitamin K_1 deficiency, suggesting that oestrogens conserve clotting factor synthesis under those conditions (Rama Rao et al 1964; Matschiner & Willingham 1974).

It is also possible that androgens may affect vitamin K_1-dependent clotting factor synthesis. There is evidence that castrated male rats are less sensitive to the effects of vitamin K_1 deficiency than intact male rats (Matschiner & Willingham 1974). Furthermore, administration of exogenous testosterone augments the rate of onset of vitamin K_1 deficiency, both in normal male rats, and castrated female rats (Uchida et al 1985). However, there is little information on the influence of either castration or sex steroid hormones on the action of coumarin anticoagulants. Therefore, we have investigated the action of *S*-warfarin in untreated castrated rats and in castrated rats given either 17β-oestradiol or testosterone.

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Materials and methods

Male Wistar rats (n = 36), 4 weeks old, were anaesthetized (Hypnorm; 0·3 mg kg^{-1} fentanyl citrate, 10 mg kg^{-1} fluanisone i.m.), and were then either castrated (n = 26), or underwent sham operation (n = 10). After recovery, they were left for 6–10 weeks before further experimentation.

At least seven days before the administration of sex steroid hormones, blood samples (1·0 mL max) were taken from the tail artery of each rat (lightly anaesthetized with diethyl ether) into trisodium citrate (10% by volume), and kept on ice before assay. Plasma testosterone concentrations were measured by radioimmunoassay (Amersham International, UK).

To assess the effect of warfarin in castrated rats pretreated with vehicle, and in sham-operated rats, eight vehicle-treated castrates, and 10 sham-operated rats were used. Blood samples (0·6 mL max) for the measurement of prothrombin time and prothrombin complex activity (PCA) were collected from the caudal artery as above and prothrombin time determined by the method of Quick (1957), using a Schnitger & Gross automatic coagulometer. PCA (%) was related to prothrombin time by reference to standard curves derived from either intact or castrated rats (as appropriate). The PCA of plasma used for the standard curve was determined by accurate dilution of normal plasma with plasma from severely anticoagulated rats (clotting times > 500 s, indicating the absence of vitamin K_1-dependent clotting factor precursors).

Both sham-operated and castrated rats were then given polyethylene glycol 200 (vehicle) (0·4 mL kg^{-1} day^{-1} i.p.), and further blood samples were collected 24 h and 7 days later. The vehicle had no significant effect on PCA in normal rats. After 7 days pretreatment, *S*-warfarin (6·3 mg kg^{-1} i.p.) was given to each rat, and blood samples were taken 6, 12, 24, 48 and 72 h later.

Three groups of castrated rats were subjected to the protocol described above, except that 17β-oestradiol (50 μg kg^{-1} day^{-1} i.p., n = 4; or 500 μg kg^{-1} day^{-1} i.p., n = 6), or testosterone (500 μg kg^{-1} day^{-1} i.p., n = 8) were given in place of vehicle.

Twenty-four hours after the administration of warfarin, an additional blood sample (1·0 mL max) was taken, centrifuged (3000*g* for 4 min), and plasma warfarin concentrations determined by normal-phase

high performance liquid chromatography (Shearer 1983).

The drugs used were: diethyl ether (analytical grade, BDH, Poole, UK); Hypnorm (Crown Chemical Co., Lamberhurst, UK); 17β-oestradiol, testosterone propionate (Sigma, Poole, UK); *R,S*-warfarin (Sorex Laboratories, Widnes, UK).

All results are expressed as mean ± s.e. mean. Statistical significance was determined using the Mann-Whitney U-test for non-parametrically distributed data.

Results

Plasma testosterone concentrations were measurable in all sham-operated rats, but there was considerable intra-individual variation (178 ± 43 pg mL^{-1}). In contrast, testosterone levels in all but one of the castrated rats were below the limits of detection (≤ 6 pg mL^{-1}; Amersham, UK); one had a level of 26·0 pg mL^{-1} and was excluded from further study. Since cross-reactivity of the present assay to dihydrotestosterone appears to be low in intact male samples, it seems unlikely that our results were influenced by dihydrotestosterone.

There was no difference between resting prothrombin times after either vehicle or steroid pretreatment in castrated rats (17·6 ± 0·5 s in vehicle-pretreated, 18·3 ± 0·9 s in oestradiol-pretreated, and 17·2 ± 0·3 s, in testosterone-pretreated rats). When these data were pooled, the resting prothrombin times of the castrated rats (17·9 ± 0·4 s) were significantly shorter ($P \leq 0·01$) than those in sham-operated rats (19·1 ± 0·5 s). To normalize this difference, the effects of warfarin have been expressed in terms of prothrombin complex activity (PCA), relative to resting levels of either castrated or sham-operated rats, respectively.

Following warfarin treatment, there was a significant fall in PCA in both groups of rats (Fig. 1). PCAs measured 6 h after warfarin were not different in either group. However, by 12 h, the PCA of sham-operated rats had fallen to a level significantly ($P \leq 0·05$) lower than that in the vehicle-pretreated castrated rats, a difference sustained at 24 h ($P \leq 0·05$). PCA subsequently recovered in both groups. The significant difference between the groups was sustained after 48 h ($P \leq 0·05$), but after 72 h, PCA was not different.

Warfarin caused a decline in PCA in both 17β-oestradiol- and testosterone-treated rats (Figs 1, 2). The initial fall was the same in each group, and between 0–12 h it was not different from that seen in vehicle-treated, castrated rats.

Twenty-four hours after warfarin, the fall in PCA was at its greatest in all groups, the PCA in rats given 17β-oestradiol being the same as that seen after vehicle alone, while that of rats given testosterone was significantly ($P \leq 0·05$) lower than in vehicle-treated rats, but remained higher than that in sham-operated rats ($P \leq 0·05$).

PCA then recovered so that there were no significant differences between the 17β-oestradiol-treated rats and those given testosterone. Recovery was also not different from that seen in the vehicle-treated, castrated rats.

At the time at which PCA was lowest in all of the groups (24 h after warfarin was given), plasma levels were between 0·75 and 2·04 μg mL^{-1} with no significant differences between the groups.

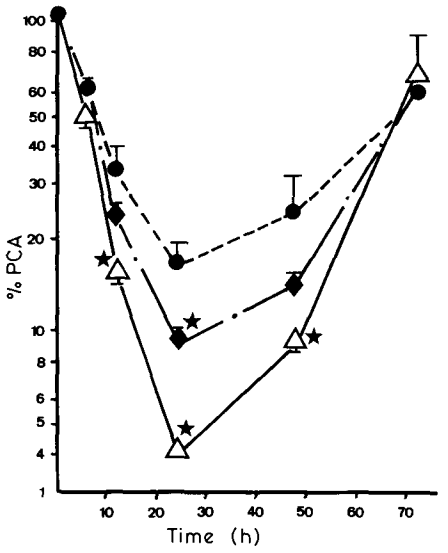

FIG. 1. Percent prothrombin complex activity (PCA) over the first 72 h after administration of warfarin (6·3 mg kg^{-1} i.p.) in castrated male rats given vehicle (●), or 500 μg kg^{-1} day^{-1} testosterone (◆), and in sham-operated rats (△). Asterisks denote statistical significance between the groups (Mann-Whitney U-test; $P \leq 0·05$).

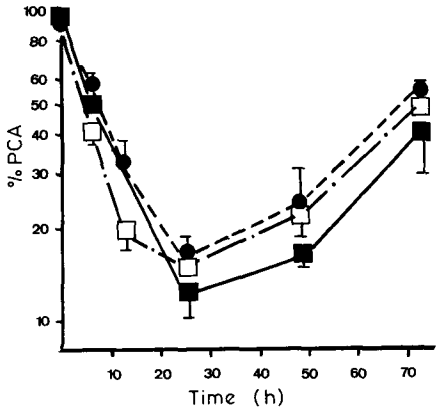

FIG. 2. Percent prothrombin complex activity (PCA) over the first 72 h after administration of warfarin (6·3 mg kg^{-1} i.p.) in steroid-pretreated castrated rats given either: (●) vehicle (0·1 mL kg^{-1} day^{-1} i.p.), (■) 17β-oestradiol pretreatment (50 μg kg^{-1} day^{-1} i.p.), (□) 17β-oestradiol pretreatment (500 μg kg^{-1} day^{-1} i.p.).

Discussion

The resting prothrombin times of castrated male rats were significantly shorter than those in intact male rats. The data are similar to the findings of Matschiner & Willingham (1974). These findings do not appear to be due only to the removal of the influence of testosterone in male rats, since its administration had no effect on resting prothrombin time. Similarly, the difference between castrated and intact rats did not appear to be due to the unmasking of an influence of oestrogens in the castrated rats, since 17β-oestradiol did not affect their resting prothrombin times. Moreover, resting prothrombin times in intact male and female rats from these laboratories were not different (Winn et al 1987) so it appears that the differences between intact and castrated rats can be attributed to castration itself.

One possible explanation of these findings is that the hepatic concentration of vitamin K_1 is higher in castrated than in intact rats which may, in turn, promote enhanced synthesis of vitamin K_1-dependent clotting factor precursors. Although this possibility was not addressed directly by these experiments, we (Winn et al 1987) and others (Haroon & Hauschka 1983) did not find significant differences between the hepatic levels of vitamin K_1 in male and female rats despite male rats being the more sensitive to coumarin anticoagulation (Winn et al 1987). An alternative explanation is that changes in the plasma concentrations of hypothalamic and adenohypophyseal hormones, caused by castration, have direct and significant effects on clotting factor synthesis (Landaburu et al 1971; Kita & Lamartiniere 1981).

The present results also showed castrated male rats to be less sensitive than intact male rats to the effects of warfarin. These data are consistent with evidence from vitamin K-deficient rats, where castration significantly delayed the onset of anticoagulation in male rats (Matschiner & Willingham 1974; Uchida et al 1985). Interestingly, we also found that testosterone significantly increased the sensitivity of castrated rats to warfarin. But, this did not appear simply to be due to testosterone reversing the effect of castration (see above). That testosterone enhanced the metabolism and elimination of warfarin seems unlikely, as plasma concentrations of warfarin were not significantly different in any of the rats 24 h after its administration.

In contrast with earlier work in vitamin K deficient rats, there was no significant difference between the effect of warfarin in rats given vehicle, or 17β-oestradiol at either dose. The difference between the reported response of vitamin K-deficient rats and our warfarin-treated rats may indicate that oestradiol influences clotting factor synthesis at a site remote from the site of action of coumarin anticoagulants (i.e. distal from vitamin K_1 epoxide reductase and vitamin K_1-reductase; Whitlon et al 1978). Indeed, this suggestion is supported by Jolly et al (1977) who showed that administration of oestradiol augmented the uptake of vitamin K_1 from the gastrointestinal tract of the rat.

In conclusion, we have shown in male rats that castration both reduced resting prothrombin times, and the sensitivity to warfarin, but there was no evidence to suggest that 17β-oestradiol protected against the action of warfarin in the castrated animals.

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