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Effect of tyramine on the atrium and the papillary muscle of the immunosympathectomized rat

SIR,—Burn & Rand (1958) proposed that tyramine exerted its sympathomimetic effects through the release of endogenous noradrenaline. This hypothesis is now generally accepted (Trendelenburg, 1963; Muscholl, 1966), although evidence for a direct action of tyramine has also been reported (Luduena, 1963; Varma & Benfey, 1963; Varma, Gillis & Benfey, 1964; Zaimis, 1965; Krzanowski & Woodbury, 1966). Most experiments on the mode of action of tyramine have been made after depleting noradrenaline stores by reserpine or by surgical denervation. Since immunosympathectomy can produce almost complete destruction of the peripheral sympathetic nervous system (Levi-Montalcini & Angeletti, 1962; Zaimis, 1965; Iversen, Glowinski & Axelrod, 1966), we examined the effect of tyramine on the myocardium of immunosympathectomized rats.

Immunosympathectomy was produced by subcutaneous injection of 0·2 ml of 61,000 anti-units/ml of bovine anti-serum to nerve-growth factor (kindly supplied by Dr. R. K. Richards, Abbot Laboratories, Chicago) in Sprague-Dawley rats within 24 hr of birth. The effectiveness of this treatment in producing immunosympathectomy was described by Iversen & others (1966) and confirmed by us (Varma, 1967) and also during the present experiments. Rats were used approximately 3 months after birth. A group of normal and immunosympathectomized rats were also injected subcutaneously with 1 mg/kg of reserpine one day before the experiment. Each rat was killed by a blow on the head and the heart rapidly excised. Atria were removed, freed of ventricular tissue and set up in a 100 ml organ bath containing Krebs-Henseleit solution at 37° and aerated with a mixture of oxygen 95% and carbon dioxide 5%. Spontaneous contractions were recorded by a Grass force-displacement transducer on a Gilson polygraph. Tension on the atria was adjusted to give maximum contraction. This was approximately 0·5 g. Papillary muscle was removed from the left ventricle and set up in a separate 100 ml organ bath under identical conditions. The muscle was stimulated by square wave pulses of 5 msec duration at 1 c/sec and supramaximal voltage. A Tektronix stimulator was used. Both preparations were allowed to stabilize for at least 1 hr during which period the bath fluid was changed several times. Cumulative concentration-response curves to tyramine were determined. Preparations were then washed repeatedly for 1 hr after which cumulative concentration-response curves to noradrenaline were determined. Initial concentration of tyramine hydrochloride was 0·01 μg/ml and that of noradrenaline bitartrate monohydrate 0·001 μg/ml. Concentrations were increased by a factor of about 3 and the next highest concentration was

added after the effect of the preceding concentration had reached a plateau. Significance of the difference between the responses of the experimental and control preparations was calculated according to Dunnett's procedure (Dunnett, 1955). Significance of difference between two means was tested by Student's *t* test. Doses of tyramine and noradrenaline refer to the salts used.

Control atrial rates of normal rats and reserpine-treated rats were 231 and 200 beats/min, respectively. This difference was not significant. The control rates of the atria of immunosympathectomized rats and immunosympathectomized reserpine-treated rats were 234 and 180 beats/min, respectively. This difference was significant ($P > 0.01$). There was no significant difference in the control contractile force of the atria and the papillary muscles in these four groups. The inotropic and the chronotropic effects of tyramine and noradrenaline are presented in Fig. 1. The chronotropic effect of tyramine on the atria of immunosympathectomized rats was not significantly different from that on the atria of control rats. However, the chronotropic effect of tyramine on the atria of reserpine-treated rats was significantly reduced ($P > 0.05$). Both reserpine and immunosympathectomy produced significant reduction in the positive inotropic effect of tyramine on the atria and the papillary muscles ($P > 0.01$). A reduction in the inotropic effect of tyramine on the atria of immunosympathectomized rats has been reported earlier (Varma, 1967). The chronotropic effects of noradrenaline were generally greater in preparations from reserpine-treated or immunosympathectomized animals but the differences were not significant.

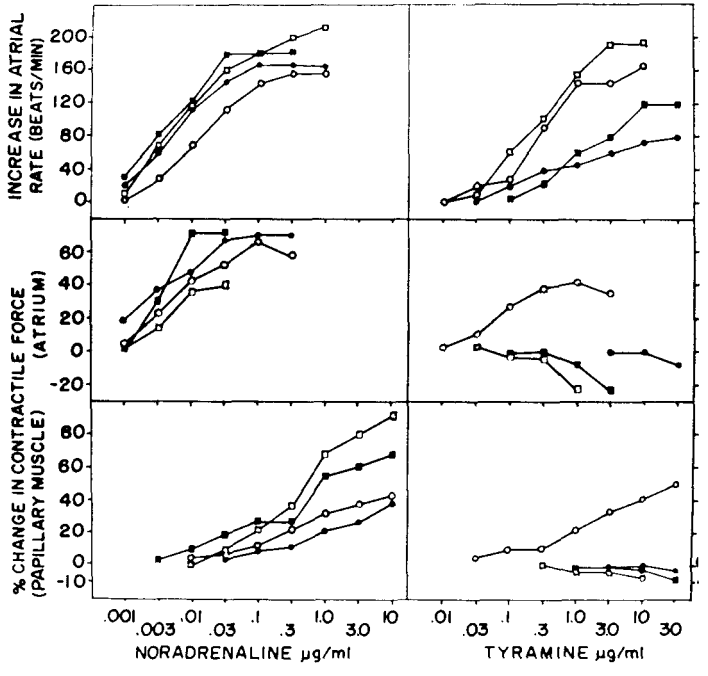

FIG. 1. Response of the isolated atria and papillary muscle of rats to noradrenaline and tyramine. ○—controls; ●—reserpine treated; □—immunosympathectomized; ■—immunosympathectomized + reserpine treated. Reserpine, 1 mg/kg, was injected (subcutaneously) one day before the experiment. Three to 6 animals in each group.

These experiments clearly show that depletion of myocardial noradrenaline by immunosympathectomy does not reduce the effect of tyramine on the atrial pacemaker. Zaimis (1965) reported that immunosympathectomy produced marked depletion of cardiac noradrenaline but did not inhibit the responses to tyramine and concluded that tyramine has a direct sympathomimetic action. Our results support this conclusion. It is interesting to note that although the chronotropic effect of tyramine is not inhibited by immunosympathectomy, the inotropic effect is significantly reduced. It seems that the inotropic and chronotropic effects are governed by different processes. It is not unlikely that these two effects are produced by activation of different receptors.

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Protamine-induced hypocalcaemia in rats

SIR,—During a clinical trial of the antitumour agent, Prolothan G (an aqueous solution of protamine standardized to contain nitrogen 2·5% w/v with dextrose 40%), some patients developed tetany and almost all became hypocalcaemic (Anderson, Tomlinson & Wright, 1967). The known neutralizing effect of protamine sulphate on heparin was therefore suspected. Heparin enhances the action of parathyroid hormone on bone resorption *in vitro* (Goldhaber, 1965) and may cause osteoporosis in man (Griffith, Nichols & others, 1965). We have now examined the effect of Prolothan G and compared it with clupeine sulphate and thyrocalcitonin in rats.

Male albino Wistar rats, 150 g, were anaesthetized with ether and a polythene cannula was inserted in the right femoral vein. Solutions were infused over an 80 min period at a rate of 0·5 ml/hr. Blood samples were taken from the tail vein (Sandiford, 1965) before, and at 20 min intervals throughout the infusion. The plasma calcium concentration was measured in 0·05 ml of plasma (MacIntyre, 1957) with the Optica CF4 spectrophotometer and flame attachment. The solutions of Prolothan G (Duncan, Flockhart & Evans), clupeine sulphate (B.D.H.) and thyrocalcitonin (M.R.C. calcitonin standard A) were prepared in physiologically normal dextrose saline. The protein estimation of the solutions was by the method of Lowry, Rosebrough & others (1951). Four animals were used for each infusion of Prolothan G, clupeine sulphate, thyrocalcitonin and dextrose saline.