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Direct and indirect effects of ephedrine on heart rate and blood pressure in vehicle-treated and sympathectomised male rats

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ABSTRACT

We have investigated the cardiac and pressor responses to (±)-ephedrine and (-)-ephedrine in pentobarbitone anaesthetized male wistar rats. The tachycardiac responses to (±)- and (-)-ephedrine were similar, but pressor responses to (-)-ephedrine (10 mg/kg) were significantly greater than those to (±)-ephedrine, and for both, the pressor response was followed by a small depressor response. Sympathectomy did not affect pressor responses, but significantly increased the later depressor response to both compounds. Sympathectomy did not affect tachycardiac or depressor responses to the β-adrenoceptor agonist isoprenaline, but significantly reduced the tachycardia to (±)-ephedrine. (±)-Ephedrine contracted vas deferens from vehicle treatment animals, but in vas deferens from sympathectomised rats, (±)-ephedrine produced almost no tonic contraction (α_{1A}-adrenoceptor mediated), but the phasic contraction was unaffected (α_{1D}-adrenoceptor mediated). It is concluded, firstly, that (-)-ephedrine is more potent than the racemate mixture at producing pressor responses. Secondly, since the depressor response to isoprenaline was unaffected, sympathectomy presumably reduced a pressor component to the response to (±)- and (-)-ephedrine. Hence, a component of the pressor response to both (±)- and (-)-ephedrine is indirect and may involve actions at α_{1A}-adrenoceptors, at which ephedrine does not have marked direct actions.

1. Introduction

Sympathomimetic amines such as ephedrine can act by directly stimulating adrenoceptors or indirectly by release of noradrenaline (NA) (see Docherty, 2008). Previous studies in anaesthetized rats and isolated tissues have suggested that ephedrine has a mixture of direct and indirect actions on blood pressure in anaesthetized rats (Kobayashi et al., 2003), or that the blood pressure actions are mainly direct (Liles et al., 2006). Liles et al. (2007) employed mice lacking the dopamine-β-hydroxylase gene (DBH-KO), preventing formation of NA from dopamine, to answer this question. The rises in blood pressure to the archetypal indirect sympathomimetic tyramine were virtually abolished in DBH-KO mice, demonstrating that tyramine acted virtually exclusively by an indirect mechanism in these studies (Liles et al., 2007). The α-adrenoceptor-mediated peak pressor response to ephedrine, like those to the direct agonists NA and phenylephrine, was unaffected by DBH-KO. Hence, the conclusion from the above study was that the actions of ephedrine are directly mediated, at least in the mouse and in terms of blood pressure.

After many years of use of this agent, there is still confusion as to the exact actions of ephedrine: direct agonism, and at which receptor subtypes, or indirect actions as an indirect sympathomimetic. We have studied the effects of chemical sympathectomy with 6-hydroxydopamine on responses in anaesthetized rats and isolated tissues from the rat to attempt to clarify the indirect actions of ephedrine.

2. Materials and methods

Male Wistar rats (230–300 g) were obtained from Envigo (UK). All studies have been approved by the Health Products Regulatory Agency (HPRA) in Ireland, pursuant to Part 8 of the European Union (Protection of Animals Used for Scientific Purposes) Regulations 2012 (S.I. No. 543), and by the RCSI Research Ethics Committee. The animals were housed in a controlled environment with a 12-h light, 12-h dark cycle and were fed a standard rat diet.

2.1. Pretreatment with 6-hydroxydopamine

6-OHDA was weighed out freshly and dissolved in ascorbic acid (1 mg/ml) immediately prior to injection. Animals were injected with

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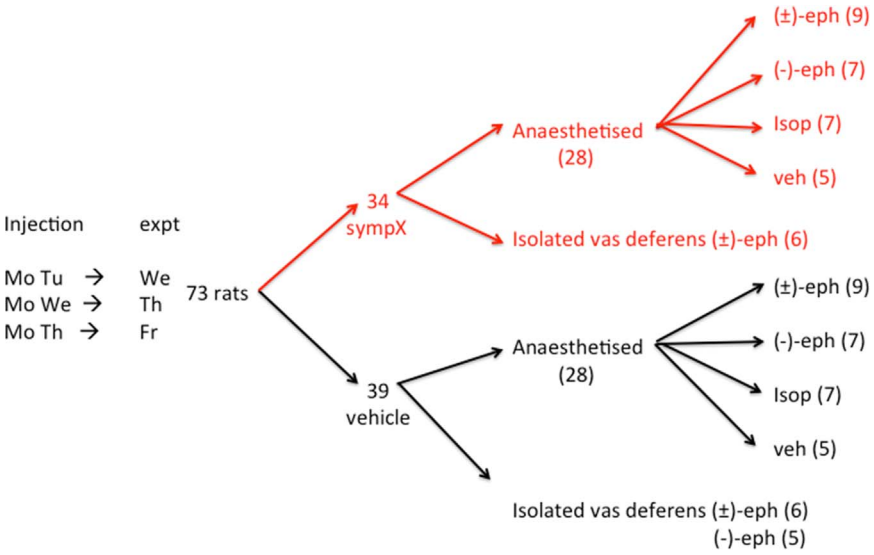

Fig. 1. Diagrammatic representation of the experimental protocol. Animals were typically given the first injection on Monday (Mo) and second injection on Tuesday (Tu) Wednesday (We) or Thursday (Th), with the experimental day, the next day: We, Th or Friday (Fr). Animals were divided into two groups, sympathectomised (sympX) and vehicle, and two kinds of experiment were carried out, anesthetized rat and isolated vas deferens. Drugs investigated were (±)-ephedrine ((±)-eph), (-)-ephedrine ((-)-eph) and isoprenaline (isop) and vehicle (veh). Numbers indicate number of animals/experiments.

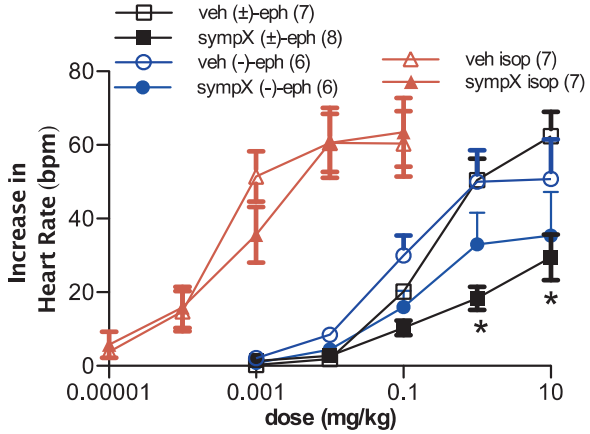

Fig. 2. Effects of intravenous injection of (±)-ephedrine ((±)-eph), (-)-ephedrine ((-)-eph) (0.001–10 mg/kg), or isoprenaline (isop) (0.00001–0.1 mg/kg), on heart rate in pentobarbitone anaesthetized vehicle-treated (veh) or chemically sympathectomised (sympX) male rats. Error bars indicate S.E.M. from 6 to 8 experiments. Asterisks denote responses to (±)-ephedrine in sympathectomised rats significantly difference from responses in vehicle-treated animals (* $P < 0.05$, two way anova and Bonferroni test).

6-OHDA (40 mg/kg, i.p.) in ascorbic acid or with vehicle (ascorbic acid 1 mg/kg, i.p.) once per day on two different days, and employed in studies the day following the last injection. Animals were injected on day 1 but the subsequent second injection was given either on day 2, 3 or 4, to produce a chemical sympathectomy, and animals were investigated on the following day (day 3, 4 or 5). This treatment schedule allowed injections beginning on Mondays, with second injection on Tuesday, Wednesday or Thursday, and experimental days on Wednesday, Thursday or Friday (see Fig. 1). There was no evidence from this study or from our previous studies that the day of the second injection affected the degree of sympathectomy. Chemically sympathectomised rats were compared with ascorbic acid vehicle treated animals.

2.2. Cardiovascular studies in anaesthetized rats

Rats (28 vehicle treated and 28 sympathectomised; see Fig. 1) were anaesthetized with pentobarbitone sodium (60 mg/kg, i.p., and maintenance doses, in volumes of 0.1 ml of 9 mg/kg, as required, i.v.). A midline incision was made in the neck and the carotid artery and jugular vein were exposed by blunt dissection, and cannulated for recording of blood pressure, and for injection of drugs, respectively. The carotid artery cannula contained heparinised saline (heparin sodium 50 I.U./ml) and the jugular cannula contained normal saline (NaCl 0.9 g/100 ml). Diastolic blood pressure (DBP) was measured using a Sensonor 840 blood pressure transducer (Sensonor, Horten, Norway) and heart rate (HR) was extracted from blood pressure. Animals were placed on a Harvard Heated Small Animal Table for maintenance of body temperature. At the end of the experiment, animals were killed by overdose of anaesthetic (i.v.), cervical dislocation and exsanguination.

Once the blood pressure and HR recording had stabilized (usually within 15 min), saline vehicle was injected intravenously in a dose of 1 ml/kg and flushed in with a volume of 0.5 ml/kg saline, in vehicle-treated and chemically sympathectomised male rats. Dose-response curves were constructed for (±)-ephedrine (0.001–10 mg/kg) (n = 9 per group), (-)-ephedrine (0.001–10 mg/kg) (n = 7 per group), and isoprenaline (0.00001–0.1 mg/kg) (n = 7 per group) (see Fig. 1), given cumulatively in 1 log unit increments, or to multiple additions of vehicle (n = 5 per group), at 2 min intervals intravenously, beginning 5 min after injection of saline vehicle. Peak changes in DBP and HR were measured. For ephedrine (10 mg/kg), the complete time course of change in DBP in the 2 min following injection was calculated. For a total of 5 rats, complete cardioaccelerator dose response were not obtained so that these experiments were omitted from dose-response curves (Figs. 2–4), but included in the time course of the blood pressure response to ephedrine (10 mg/kg) (Fig. 5).

2.3. Preparation of the isolated rat vas deferens

Animals were killed by overdose of anaesthesia with pentobarbitone, cervical dislocation and exsanguination.

For removal of vas deferens, a midline incision was made in the abdomen and the testis and epididymis exposed. Blunt forceps were placed to separate the vas deferens from the connective tissue. The whole vas deferens was tied with a long thread at the epididymal end and a short thread at the prostatic end, removed from the rat, and carefully cleared of connective tissue and blood vessels. By convention, the vas was always placed in organ baths with the prostatic end attached to a fixed rod (see Docherty, 1979), and the epididymal end attached to a transducer (Grass FT03) under 1 g tension in organ baths at 37 °C in Krebs-Henseleit solution of the following composition: (mM): NaCI 119; $NaHC0_3$ 25; D-glucose 11.1; KCl 4.7; $CaCl_2$ 2.5; KH_2P0_4 1.2; $MgS0_4$ 1.0.

Bathing fluid was changed every 15 min, except during dose response curves. Following 30–45 min equilibration, tissues were contracted with NA (10 μM), and washed. Bathing fluid was again changed