Direct recording of arterial blood pressure and heart rate in the conscious rat

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Several techniques for recording the arterial blood pressure of conscious rats have been described (Weeks & Jones, 1960; Popovic & Popovic, 1960; Buckingham, 1976). We have modified and developed the techniques of Popovic & Popovic (1960) to enable us to measure blood pressure and heart rate for several hours without seriously restricting the movement of the rats. A method of inducing rats voluntarily to consume oral solutions of drugs whilst blood pressure is being recorded is also described.

Construction of cannulae. Blood pressure was recorded from a cannula inserted into the carotid artery. Arterial cannulae were made from polyethylene tubing i.d. 0·35 mm, o.d. 1·05 mm (Dural plastics, SP19). Cannula tips were fashioned by firmly holding the tubing approximately 15 cm from the free end of a roll of tubing and rapidly pulling it to form a fine point with about half the original external diameter. The length of the polyethylene was then cut off square about 1–2 mm from the point of constriction. This type of blunt cannula is easily inserted into the carotid artery and remains patent much longer than a cannula with a bevelled tip. The distance from the point of insertion of the cannula to the aorta was determined in dead rats. Approximate distances were as follows: 120 g rat 14 mm, 200 g rat 17 mm, 300 g rat 19 mm. The appropriate distance was marked on the cannula before insertion. It was found that this measure was sufficient to ensure that the cannula tip lay in the aortic arch, so that a 'knee' in the cannula, as described by Popovic & Popovic (1960), was unnecessary. The 'knee' makes insertion of the cannula much more difficult.

Insertion of cannulae. Animals were anaesthetized with ether. The carotid artery was exposed by blunt dissection, a ligature was tied at the distal end of the carotid artery, and a second placed loosely about the middle of the exposed area of artery. The inflow to the artery was stopped by a soft wire retractor inserted beneath the vessel; the retractor was formed from a piece of malleable wire with tips covered with soft silastic rubber to prevent damage to the vessel wall. (In our experience small animal artery clamps initiated a necrotic reaction resulting in early cannula failure). A small incision was made in the vessel as close as practicable to the anterior ligature. The cannula (filled with 1% heparin in saline) was inserted and slowly fed into the artery to the point marked on the polyethylene. The ligature previously left loose was firmly tied and a further ligature was tied caudal to the first.

The cannula was then checked for patency, as described by Popovic & Popovic (1960). If, after induction of a slight negative pressure with a syringe, blood flow and a strong pulse were not discernible in the cannula, it was withdrawn a few millimetres until a strong pulse developed. Exteriorization of the cannula was as described by Popovic & Popovic (1960), with the cannula passing from the neck incision under the skin between the left eye and ear to emerge at a point along the midline of the body slightly posterior to the ears. The free end was stoppered with a short length of tapered 22 gauge steel wire.

Recording of blood pressure. A piece of 22 gauge steel cannula tubing was used to connect the cannula to a 40 cm long polyethylene lead, i.d. 1 mm, o.d. 2 mm (SP 74, Dural plastics) running to a Statham P23 AA pressure transducer. Polyethylene tubing of smaller diameter was less successful. A closely coiled spring 8 cm long was secured about the polyethylene lead 4 cm from the free end to prevent the rats biting through it. Blood pressure was recorded by a Servoscribe RE 520.20 pen recorder. During recording 0·9% NaCl solution containing heparin 1% was infused into the cannula at a rate of 0·37 ml h^{-1} by means of a Sage 341 infusion pump, to prevent clotting of blood cells in the tip of the cannula. While blood pressure was being measured the rats were placed in a metal cage, 14·5 by 24 cm which was large enough for the rat to move freely, but small enough for the polyethylene tube not to become too readily tangled. Occasional surveillance of the rat during recording was required.

The time for which cannulae remained patent depended on their usage. If daily readings were taken cannula failure often occurred after seven to ten days due either to the cannula being pulled up the carotid or to severing of the carotid artery at the ligature of cannula and artery. When readings were taken less frequently cannulae remained patent for up to thirty days. Daily flushing of cannulae was not necessary to maintain patency, nor was additional anticoagulant therapy required.

Administration of drugs. Rats were induced by training to consume a drug solution in a single dose over five to ten minutes. They were isolated in cages three to four days before arterial cannulation, and allowed free access to food and water during the day but water bottles were removed overnight. In the morning 4–5 ml of a milk substitute (Complan) were placed in a glass centrifuge tube fitted with a rubber bung and drinking nozzle, and the tube positioned above the cage using a retort stand. As the rats became accustomed to receiving fluid in this manner, they readily accepted it from a tube held manually. Drugs were added to the milk substitute as

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required and blood pressure and heart rate could be monitored before, during and after ingestion of the drug for up to 4 h.

We have found this technique for insertion of arterial cannulae to be quick and reliable. Animals appear to experience little trauma from the operation, and are ready to be used the following day. The time and materials required to fashion the cannula are considerably less than that described by Popovic & Popovic (1960). The constriction formed at the end of the cannula permits easy insertion into the artery, while the main body of the cannula remains of a fairly large diameter facilitating connection procedures and the recording of heart rate. The main advantage of this method is that the cardiovascular effects of drugs can be evaluated in conscious rats in a familiar and non-restrictive environment.

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An analysis of the inhibitory effects and of possible prostaglandin antagonism of steroid sex hormones in the guinea-pig ileum

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Progesterone, pregnenolone, testosterone, ethinyloestradiol, oestrone, oestriol reversibly inhibit guinea-pig isolated ileum contractions to acetylcholine, histamine (Seaman, Famaey & others, 1977a), nicotine and 5-hydroxytryptamine (5-HT) (Seaman, Fontaine & others, 1977b) and these inhibitions could be reversed by prostaglandins (PG) E_1 or $F_{2\alpha}$. It was concluded that these steroids exert an overall spasmolytic effect on ileal smooth muscle. This was also the conclusion of Ishida, Oshima & others (1972) who noted a papaverine-like action of some sex hormones on the ileum.

Pregnenolone, testosterone, ethinyloestradiol and oestriol also caused a more specific inhibition of contractions induced by nicotine, an indirect agonist, or 5-HT a partly indirect agonist (Seaman & others, 1977b). Similar actions were observed by us with non-steroidal anti-inflammatory drugs (NSAID) (Famaey, Fontaine & Reuse, 1977a; Famaey, Fontaine & others, 1977c) and anti-inflammatory steroids (AIS) (Famaey, Fontaine & Reuse, 1975b; Famaey, Fontaine, Seaman & Reuse, unpublished) and were attributed partly to the effects of these drugs on biological membranes and partly to their effects on PG production.

PG are themselves partly direct (Bennett & Fleshler, 1970) and partly indirect agonists (Bennett, Eley & Scholes, 1968; Bennett, Eley & Stockley, 1975) on the ileum and we found high concentrations of NSAID and AIS to have a preferential antagonism towards PGE_1 and $F_{2\alpha}$-induced contractions compared with acetylcholine contractions (Famaey, Fontaine & Reuse, 1977b).

We have now investigated whether similar antagonism occurs with steroid sex hormones.

Submaximal contractions (as determined by dose-action curves) of the longitudinal muscle of the guinea-pig isolated ileum were elicited by PGE_1 (5 ng ml^{-1}) or $PGF_{2\alpha}$ (20 ng ml^{-1}) (45 s contact time, every 6 min) on ileal segments (4 cm length, removed at least 10 cm from the caecum) set up in Krebs–Henseleit solution at 37° and gassed with a mixture of 5% CO_2 in oxygen.

The hormones were added to the bath after three reproducible control contractions to PG and the ileum was again challenged with the PGs at the same intervals. After 12 min contact the hormones were washed out and two more PG doses were added.

At concentrations of the hormones similar to those used previously by Seaman & others (1977a, b) for inhibiting contractions to acetylcholine, histamine, nicotine and 5-HT we obtained after 12 min contact significant (Student's *t*-test for paired data) inhibitions of contractions to PGE_1 and $F_{2\alpha}$ which appeared to be almost totally reversible after washing out (Table 1). Except for testosterone which inhibited contractions to $PGF_{2\alpha}$ significantly (Student's *t*-test) more than to PGE_1, there was no difference between the inhibitory effects of the hormones on the two PGs.

Pregnenolone exerted a more pronounced effect on $PGF_{2\alpha}$-induced contractions than it did to acetyl-

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