Table 1. Half-life values of technetium activity in the rabbits injected with technetium in microemulsion or aqueous solution.

Rabbit	Microemulsion	Aqueous solution
1	132	10·6
2	151	12·5
3	57*	17·9
4	46*	12·1
5	69*	9·8
6	122	8·2
7	189	9·2

Mean ± s.d. * These animals showed a biexponential decay: half-lives reported are those of exponential equations with the same intercept and the same AUC as the (biexponential) fitting obtained.

The biexponential curve was simplified to permit statistical analysis and the half-life was computed for a monoexponential curve with the same intercept and the same area under the curve (AUC) as the original biexponential curve.

Table 1 gives half-life data for each animal.

The experimental data show that pertechnetate carried by water-oil microemulsions is released from the site of administration at a slower rate than from pertechnetate administered as an aqueous solution.

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Diuretic effect of N^G-nitro-L-arginine methyl ester in the rat

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Abstract—Intravenous infusion of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester, L-NAME (10 $\mu g\ kg^{-1}\ min^{-1}$), to anaesthetized rats produced a diuresis and natriuresis. By contrast, infusion of the same dose of N^G-nitro-D-arginine methyl ester had no effect on either urine output or sodium excretion. The effects of L-NAME were first evident 120 min after the start of infusion and by 170 min a fivefold increase in urine volume and sodium excretion was recorded. L-NAME also produced a transient fall in inulin clearance and a persistent decline in renal blood flow. These renal effects of L-NAME were associated with a gradual elevation of mean arterial blood pressure, although this only attained statistical significance, in comparison with saline-infused animals, 170 min after the start of infusion. The findings indicate the diuresis and natriuresis evoked by L-NAME in the rat is a result of a direct tubular action together with a pressure diuresis.

A study in the rat using the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) revealed the renal cortical vasculature has a relatively high basal release of NO which substantially contributes to the control and maintenance of renal cortical blood flow (Walder et al 1991). Infusion of another NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) in rats has been shown to evoke falls in renal blood flow (RBF), glomerular filtration rate, urine flow and sodium excretion (Lahera et al 1993). An antidiuretic effect of L-NAME has also been demonstrated in the dog (Salom et al 1992). In a recent study, however, using rats treated with cisplatin, we noted that infusion of L-NAME potentiated the diuretic and natriuretic effects of glycine (Li et al 1994). This observation led us to investigate the effects of L-NAME infusion on renal haemodynamics and excretory function in the anaesthetized rat. Some experiments were also conducted in which renal function was monitored during infusion of N^G-nitro-D-arginine methyl ester (D-NAME), the enantiomer of L-NAME, which is inactive against NO synthase (Graves & Poston 1993). These experiments were performed to differentiate the effect on renal function of NO synthase inhibition produced by L-NAME from any effects which amino acid infusion itself might have on renal function (Cernadas et al 1992).

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Materials and methods

Materials. L-and D-NAME, and inulin were purchased from Sigma Chemical Co., UK. [^{3}H(G)]Inulin (201 mCi g^{-1}) was obtained from DuPont NEN Research Products, UK, and its stated radiochemical purity was greater than 98%.

Experimental protocol. Male albino Wistar rats, 200–250 g, were anaesthetized with thiobutabarbitone (180 mg kg^{-1}, i.p.) and cannulae inserted into the trachea to facilitate spontaneous ventilation, the left jugular vein for saline/inulin infusion, the right femoral vein for infusion of either L-NAME, D-NAME or saline (0·9% w/v NaCl), and the right carotid artery for measurement of systemic blood pressure. Mean arterial blood pressure (MAP) was measured via a pressure transducer (Druck PDCR 75). The abdomen was opened by a midline incision and a cannula was inserted into the bladder for collection of urine and an ultrasonic perivascular flow probe (model 2SB, Transonic Systems Inc, USA) was placed around the left renal artery. The probe was connected to a small-animal flowmeter (T206 Transonic Systems Inc.) to record RBF. Body temperature was maintained at 37°C by using a rectal thermometer and heating lamps. On completion of surgery, 2 mL saline containing 0·35 μCi mL^{-1} [^{3}H]inulin were administered via the jugular vein. This solution was infused for the remainder of the experiment at a rate of 100 μL min^{-1}. Thirty minutes following surgery, an infusion via the femoral vein of either L-NAME, D-NAME (10 $\mu g\ kg^{-1}\ min^{-1}$; 10 μL min^{-1} in saline) or saline (10 μL min^{-1}) was started and maintained throughout the experiment.

Each experiment consisted of seven 20-min collection clearance periods. During preliminary experiments it was noted that L-NAME did not evoke changes in renal clearance of [^{3}H]inulin (C_{IN}) or excretory function (urine volume and Na^+ excretion) until 90 min after the start of infusion. Therefore, to minimize the number of blood samples taken, the first collection/clearance period commenced 40 min after the start of the femoral infusion. During each period, urine was collected into pre-weighed tubes, and a blood sample (0·1 mL) was taken at the midpoint of each urine collection so the C_{IN} value could be estimated. Blood samples were centrifuged and plasma separated for subsequent analysis. The erythrocytes were suspended in an equal volume of isotonic saline and transfused back into the animal.

Urine and plasma analysis. Levels of [^{3}H]inulin in plasma and urine were determined by liquid scintillation counting. Urinary concentrations of sodium were assessed by flame photometry.

Analysis of results. Results are expressed as mean ± s.e.m. Comparison of means between groups was made using one-way analysis of variance with means compared by Scheffe's test.

Results and discussion

Infusion of L-NAME at a rate of 10 $\mu g\ kg^{-1}\ min^{-1}$ evoked a diuresis (Fig. 1a) and natriuresis (Fig. 1b). This effect on excretory function was delayed, occurring approximately 2 h after the start of infusion and appears to be a result of NO-synthase inhibition, since no such diuresis or natriuresis was obtained with an equivalent infusion rate of the enantiomer D-NAME, which is devoid of NO-synthase inhibitory activity (Graves & Poston 1993). The increase in urine flow induced by L-NAME occurred at a time when C_{IN} was at control levels despite an earlier fall (Fig. 2a). However, whilst C_{IN} was at control levels during the diuresis there was a significant depression of RBF (Fig. 2b) which indicates filtration fraction was increased. An increase in filtration fraction produced by L-NAME in the rat has been noted previously (Lahera et al 1991) and indicates L-NAME produces a more marked increase in postglomerular resistance compared with preglomerular resistance.

By contrast to the present findings in which L-NAME was infused at 10 $\mu g\ kg^{-1}\ min^{-1}$, previous studies in the rat have shown that L-NAME administered either as a bolus dose of 2·5 mg kg^{-1} or infused at 50 $\mu g\ kg^{-1}\ min^{-1}$ exerted an

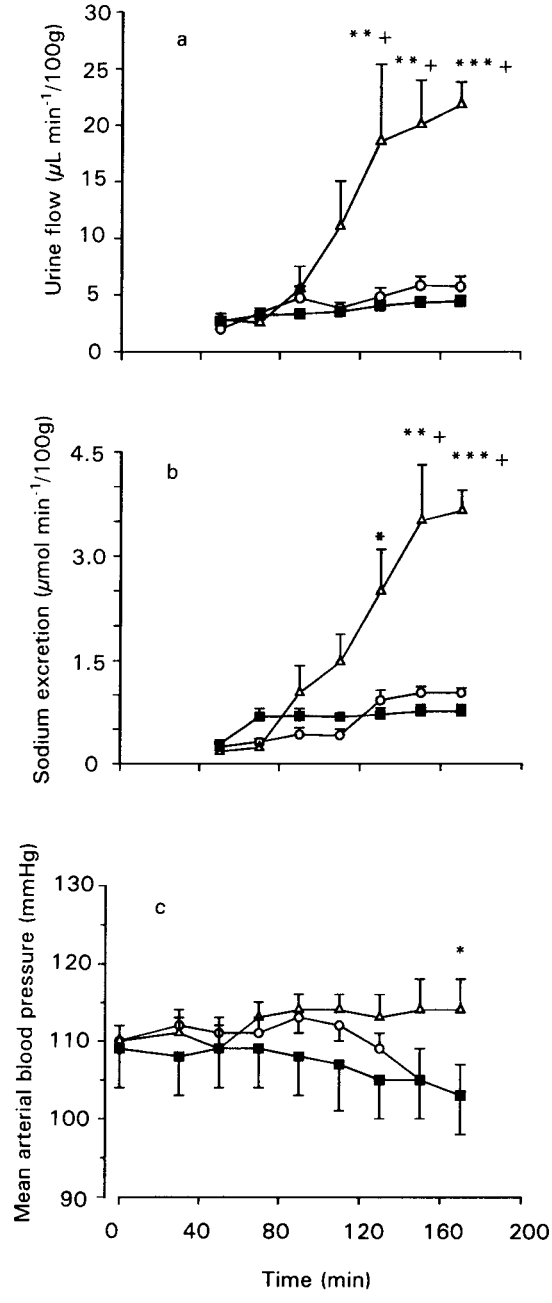

FIG. 1. Urine flow (a), sodium excretion (b) and mean arterial blood pressure (c) in anaesthetized rats infused intravenously with saline, 10 μL min^{-1} (■), L-NAME, 10 $\mu g\ kg^{-1}\ min^{-1}$ (△) or D-NAME, 10 $\mu g\ kg^{-1}\ min^{-1}$ (○). Results are given as mean and vertical bars indicate s.e.m. (n = 8). Infusions commenced at 0 min. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ relative to saline-treated rats. $+P<0.05$ relative to D-NAME-treated rats.

antidiuretic and antinatriuretic effect (Chevalier et al 1992; Lahera et al 1993). However, administration of L-NMMA to rats has been reported to produce polyuria (Zatz & de Nucci 1991) and Baylis et al (1990) have found that L-NMMA and N^G-nitro-L-arginine produce a reduction in the fractional reabsorption of sodium in the rat. A further study in the rat showed that L-NAME infused at 0·1 and 1·0 $\mu g\ kg^{-1}\ min^{-1}$ produced a fall in urine flow at 120 and 60 min, respectively, following the start of infusion, with the 1·0 $\mu g\ kg^{-1}\ min^{-1}$ rate resulting in a depression of sodium excretion (Lahera et al 1991). However,

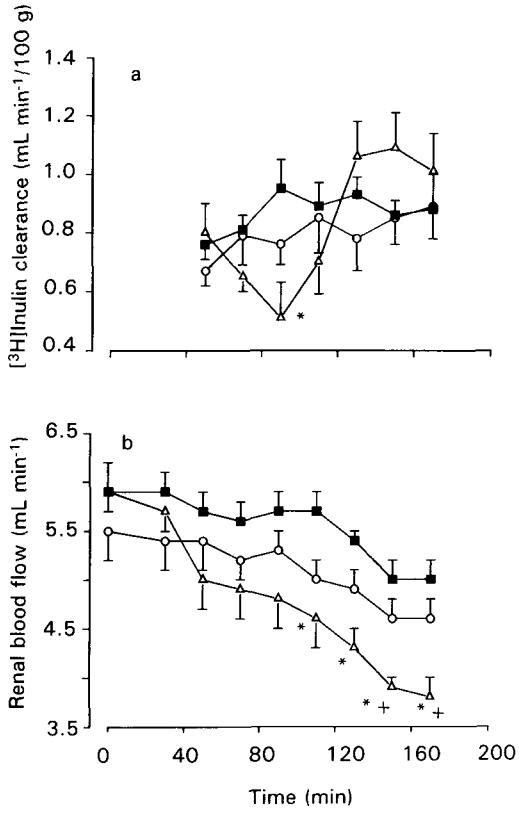

FIG. 2. [^{3}H]Inulin clearance (a) and renal blood flow (b) in anaesthetized rats infused intravenously with saline, 10 μL min^{-1} (■), L-NAME, 10 μg kg^{-1} min^{-1} (△) or D-NAME, 10 μg kg^{-1} min^{-1} (○). Results are given as mean and vertical bars indicate s.e.m. (n = 8). Infusions commenced at 0 min. *$P < 0.05$ relative to saline-treated rats. +$P < 0.05$ relative to D-NAME-treated rats.

when L-NAME was infused at 10 μg kg^{-1} min^{-1}, urine flow and sodium excretion initially fell and then returned to control levels, whilst at an infusion rate of 50 μg kg^{-1} min^{-1} a diuretic and natriuretic response was noted (Lahera et al 1991). The infusion of these higher doses of L-NAME significantly elevated MAP and as a result the ability of L-NAME to increase urine flow and sodium excretion at these higher doses was attributed to a pressure diuresis (Lahera et al 1991), the mechanism for which appears to be related to a rise in renal interstitial hydrostatic pressure (Khraibi et al 1989). In the present study, L-NAME (10 μg kg^{-1} min^{-1}) did produce a rise in MAP, although in comparison with saline-infused animals, this only attained statistical significance at 170 min (Fig. 1c). Thus it is possible that the diuresis evoked by L-NAME was a consequence of a pressure diuresis, but, after 170 min infusion, L-NAME produced, in comparison with saline-infused animals, a mean rise in MAP of 11 mmHg which was associated with a 5-fold increase in urine flow and a 4·8-fold increase in sodium excretion. By contrast, Gonzalez-Campoy et al (1989) found pressure diuresis in untreated rats resulted in a more modest increase in excretory function since a rise in renal perfusion pressure of 19 mmHg evoked a 1·8-fold increase in urine flow and a 2·3-fold increase in sodium excretion. This suggests the diuresis induced by L-NAME is, at least in part, a result of a direct tubular action. Indeed, NO synthase has been found in porcine kidney epithelial cells which are able to produce cGMP (Ishii et al 1990), and 8-bromo-cGMP, in the presence of ATP, stimulates the Na^+-H^+ antiporter in renal brush-border membranes from rabbits (Green et al 1991).

In conclusion, this study has confirmed that the NO-synthase inhibitor L-NAME can produce a diuresis and natriuresis in the rat. The results indicate that this effect on excretory function is a combination of a direct tubular action in addition to a pressure diuresis.

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