clarified if the latter actions are caused by selective blockade of dopamine autoreceptors.

This work was supported by the Swedish Medical Research Council (14X-502). Dr. Per Lindberg, Pharmacia and CIBA-Geigy generously donated drugs. Skilful technical assistance was provided by Miss Marie Lager.

May 5, 1980

REFERENCES

Andén, N.-E. (1976) in: Birkmayer, W., Hornykiewicz, O. (eds) Advances in Parkinsonism. Editiones Roche, Basle, pp. 169–177

Bunney, B. S., Roth, R. H., Aghajanian, G. K. (1975) Psychopharmacol. Commun. 1:349–358

Carlsson, A. (1975) in: Usdin, E., Bunney, Jr, W. E. (eds) Pre- and Postsynaptic Receptors. Marcel Dekker, New York, pp. 49–65

Carlsson, A., Davis, J. N., Kehr, W., Lindqvist, M., Atack, C. V. (1972) Naunyn-Schmiedebergs Arch. Pharmacol. 275:153–168

Kehr, W., Carlsson, A., Lindqvist, M., Magnusson, T., Atack, C. (1972a) J. Pharm. Pharmacol. 24:744–747

Kehr, W., Carlsson, A., Lindqvist, M. (1972b) Naunyn-Schmiedeberg's Arch. Pharmacol. 274:273–280

König, J. F. R., Klippel, R. A. (1963) The rat brain. Williams & Wilkins, Baltimore

Roth, R. H., Walters, J. R., Aghajanian, G. K. (1973) in: Usdin, E., Snyder, S. H. (eds) Frontiers in Catecholamine Research. Pergamon, New York, pp. 567–574

Rubin, A. A., Yen, H. C., Pfeffer, M. (1967) Nature (London) 216:578–579

Stock, G., Magnusson, T., Andén, N.-E. (1973) Naunyn-Schmiedeberg's Arch. Pharmacol. 278:347–361

Sugerman, A. A., Herrmann, J. (1967) Clin. Pharmacol. Ther. 8 : 261–265

Ungerstedt, U., Butcher, L. L., Butcher, S. G., Andén, N.-E., Fuxe, K. (1969) Brain Res. 14:461–471

Walters, J. R., Roth, R. H. (1976) Naunyn-Schmiedeberg's Arch. Pharmacol. 296:5–14

The depressor and renal vasodilator responses to dopamine in the rat do not depend on prostaglandin biosynthesis

M. J. ROBERTSON, N. M. HORN, B. J. CHAPMAN*, *Department of Physiology and Pharmacology, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU, U.K.*

The actions of many hormones are mediated (or moderated) by the stimulation of prostaglandin (PG) biosynthesis within the target tissue (Horton 1974). It is possible that the renal actions of dopamine (DA) in the rat might depend upon intrarenal PG synthesis. DA can have renal vasodilator actions in the rat (Chapman et al 1980a), and a number of PG's can cause renal vasodilatation (see review, Tobian 1976). DA causes diuresis and natriuresis (Chapman et al 1980b) and many PG's can similarly induce diuresis and natriuresis (Gross & Bartter 1973). Furthermore it has been asserted that some of the vascular actions of DA in the rat do depend upon local PG production (Chevillard et al 1978), and it is known that the renal actions of another catecholamine, noradrenaline, can involve the renal production of PG's (Dunham & Zimmerman 1970).

DA acts on three types of vascular receptors in the dog and man (Goldberg 1972) and in the rat (Chapman et al 1980a). Thus α-adrenoceptors mediate the vasoconstrictor, pressor responses, while β-adrenoceptors and specific dopamine receptors mediate the vasodilator, depressor responses. As cited above, it has been reported that the renal actions of noradrenaline can include an α-adrenoceptor-mediated stimulation of PG synthesis. We therefore decided to measure the vasodilator and vasodepressor actions of DA on the mean arterial blood pressure (MABP) and the renal blood flow of the rat, in the presence and absence of indomethacin (an inhibitor of endogenous prostaglandin production).

* Correspondence.

Male Wistar albino rats, 300 g, were anaesthetized with sodium pentobarbitone and tracheostomized. A femoral artery was cannulated (Portex, PP25) and connected to a pressure transducer (Elcomatic, Glasgow) and pen recorder (Vitatron U.K. Ltd. Maidenhead) for the measurement of mean arterial blood pressure (MABP). A femoral vein was cannulated (Portex, PP25) for the intravenous infusion of drugs.

The surface of the left kidney was exposed with the aid of retractors, and two H_2-sensitive Pt electrodes were inserted to different depths within the kidney parenchyma. These two electrodes were used to measure H_2 concentrations (see Haining & Turner 1966) so that blood flows could be calculated for two highly localized regions of the kidney. Calibration marks were made on the electrodes so that one electrode could be inserted to a depth of 1 mm for the measurement of cortical blood flow, and the second electrode could be inserted to a depth of 2 mm from the kidney surface for the measurement of outer medullary blood flow. A single calomel–KCl bridge, held against the exposed tissues of the leg, completed the two circuits. The preparation was then left 30 min for renal function to stabilize.

Small volumes of H_2 gas were introduced into the tracheal cannula for one or two inspirations and distributed via the blood supply to the tissues of the body. Dissolved H_2, detected by the Pt electrodes in the kidney, caused a change in electrical current through the electrode and recording system; this current was recorded on a Vitatron logarithmic recorder. The rate of change of the renal $[H_2]$ was used to calculate RBF. This method for

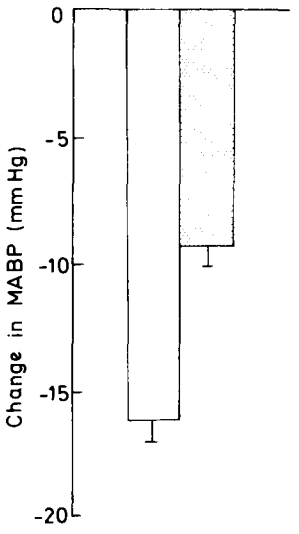

FIG. 1. The effects of arachidonic acid (300 μg kg^{-1} i.v.) on MABP before (open column) and after (stippled column) treatment with indomethacin (5 mg kg^{-1} i.v.); n = 4 rats. The difference is highly significant $P < 0.001$.

measuring renal blood flow has been described together with evidence showing its validity (Chapman et al 1980a).

In all cases, the blood flow value was calculated as the mean of two to four consecutive recordings made at 3–5 min intervals. The coefficient of variation within groups of triplicated control measurements was ± 2·6%. The vascular resistance to blood flow was calculated as the MABP divided by the blood flow and therefore has the units mmHg (ml min^{-1} per 100 g tissue)$^{-1}$.

A rectal thermometer was inserted, and body temperature maintained between 37 and 38 °C by adjusting the proximity of a lamp.

The following drugs were dissolved in 0·9% NaCl (saline) and injected or infused intravenously by means of a Braun (Melsungen) infusion pump: DA HCl (Kochlight laboratories); phenoxybenzamine (Smith Kline & French); isoprenaline HCl, arachidonic acid, indomethacin, propranolol (Sigma).

All experimental results are quoted as the mean ± s.e. of the mean, and any difference between means was evaluated using Student's t-test. The statistical methods used were those of Colquhoun (1971).

Arachidonic acid (300 μg kg^{-1}), a natural precursor of PG's, was injected i.v. into anaesthetized, but otherwise untreated rats. This caused a significant decrease in MABP (Fig. 1). It is thought that the decrease in MABP is caused predominantly by PG's synthesized from the arachidonic acid (Chevillard et al 1978). The rats were then treated with indomethacin (5 mg kg^{-1} i.v.) and the same dose of arachidonic acid retested 30 min later. Since the depressor response to arachidonate was significantly reduced 30 min after indomethacin treatment, we concluded that PG biosynthesis was effectively reduced under these experimental conditions (also, see below).

Three doses of DA (25, 50 and 100 μg kg^{-1}) were injected intravenously into rats which had been pretreated with the α-adrenoceptor blocker, phenoxybenzamine (6 mg kg^{-1} i.v.) and the changes in MABP were monitored (Fig. 2A). Dose dependent decreases in the MABP were seen, presumably due to an action on both DA-receptors and β-adrenoceptors in the peri-

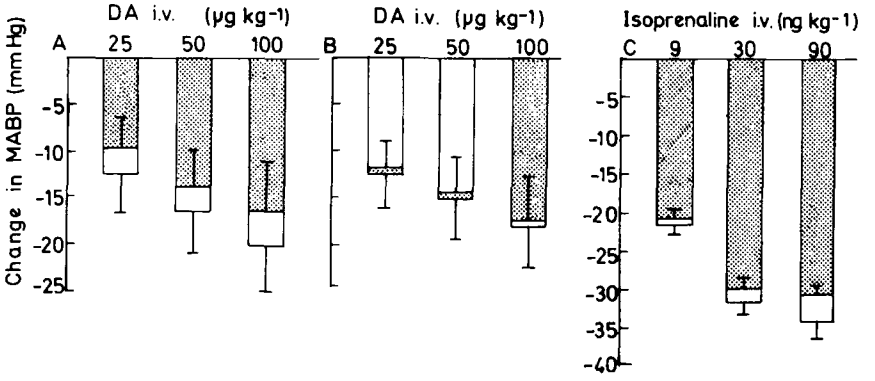

FIG. 2. A. The effect of DA on MABP before and after indomethacin treatment (5 mg kg^{-1} i.v.) in rats treated with an α-adrenoceptor blocker (phenoxybenzamine, 6 mg kg^{-1} i.v.). n = 6 rats. Open columns: phenoxybenzamine pretreatment. Stippled columns: phenoxybenzamine + indomethacin pretreatment. B. The effect of DA on MABP before and after indomethacin treatment (5 mg kg^{-1} i.v.) in rats treated with α- and β-adrenoceptor blockers (phenoxybenzamine, 6 mg kg^{-1} i.v. and propranolol, 3 mg kg^{-1} i.v. followed by 3·6 mg kg^{-1} min^{-1} i.v.); n = 6 rats. Open columns: phenoxybenzamine + propranolol pretreatment. Stippled columns: phenoxybenzamine + propranolol + indomethacin pretreatment. C. The effect of isoprenaline on MABP before and after indomethacin treatment (5 mg kg^{-1} i.v.) in rats treated with an α-adrenoceptor blocker (phenoxybenzamine, 6 mg kg^{-1} i.v.). Open columns: phenoxybenzamine pretreatment. Stippled columns: phenoxybenzamine + indomethacin treatment. All differences are not significant.

pheral circulation (Chapman et al 1980a). After indomethacin treatment, DA injections produced similar changes in the MABP which were not significantly different from the changes produced before the indomethacin treatment.

The same doses of DA (25, 50 and 100 μg kg^{-1}) were injected intravenously into rats which had been pretreated with phenoxybenzamine and the β-adrenergic receptor blocker, propranolol (3 mg kg^{-1} i.v. initially, followed by an infusion of 3·6 mg kg^{-1} h^{-1} i.v.) and the changes in MABP were monitored. The DA caused dose-dependent decreases in the MABP (Fig. 2B) due to an action on peripheral DA receptors; Chapman et al (1980a) have shown that the cardiovascular actions of DA in animals treated with α- and β-adrenoceptor blockers are mediated by specific DA receptors. All three doses of DA still caused decreases in MABP in the same animals after indomethacin treatment (5 mg kg^{-1} i.v.), and these were not significantly different from decreases seen before indomethacin treatment.

Three doses of isoprenaline (9, 30 and 90 ng kg^{-1}) were injected intravenously into rats that had been previously treated with phenoxybenzamine. Isoprenaline acts on α- and β-adrenoceptors but not DA receptors (Chapman et al 1980a); in the present study, isoprenaline was used, in the presence of phenoxybenzamine, to selectively stimulate β-adrenoceptors. The isoprenaline caused dose-dependent decreases in MABP. After indomethacin treatment, all three doses of isoprenaline caused decreases in the MABP which were not significantly different from those seen before the indomethacin treatment.

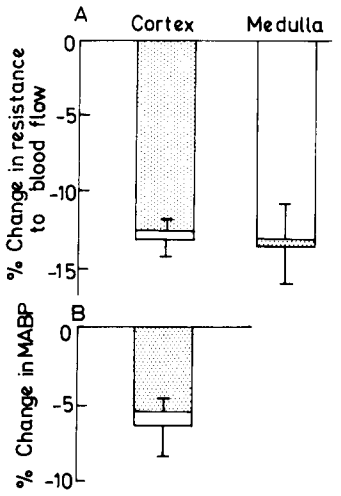

FIG. 3. The effects of DA (35 μg kg^{-1} h^{-1} i.a.) on resistance to blood flow (A) and MABP (B) in the absence (open columns) or presence (stippled columns) of indomethacin (5 mg kg^{-1} i.v.). The rats were pretreated with phenoxybenzamine (6 mg kg^{-1} i.v.) and propranolol (3 mg kg^{-1} i.v. followed by 3·6 mg kg^{-1} h^{-1} i.v.); n = 5 rats. Differences are not significant.

There are few sites in the peripheral vasculature that contain specific dopamine receptors, and the kidney is one of these (Eble 1964). We therefore decided to investigate the possible relationship between dopamine receptors and prostaglandin biosynthesis by studying localized changes in renal blood flow in the presence and absence of indomethacin. Fig. 3 shows the effect of infusing DA (35 μg kg^{-1} h^{-1}) into the renal artery of rats that were treated with the phenoxybenzamine and propranolol. This dose of DA caused increases in both the cortical and medullary blood flows and these responses to DA were not significantly altered by indomethacin. Fig. 3A shows the percentage changes in resistance to blood flow through both the cortex and the medulla of these rats; the DA infusion caused a decrease in the resistance to blood flow through both regions of the kidney, and again these responses to the DA infusion were not significantly changed 30 min after the indomethacin treatment. Fig. 3B shows the effect of the DA infusion on MABP in the same rats. The DA caused a small but significant decrease in MABP, and this decrease was also not significantly altered 30 min after treatment with indomethacin.

Before treatment with any pharmacological blocking agent, the mean blood flow through the renal cortex was 413 $\pm$ 35 ml min^{-1} per 100 g tissue and through the renal medulla was 171 $\pm$ 12 ml min^{-1} per 100 g tissue. These results compare well with those reported earlier (Aukland 1976; Chapman et al 1980a). After pretreatment with phenoxybenzamine and propranolol the blood flow values (i.e. the baseline values from which the changes shown in Fig. 3 were calculated) were 298 $\pm$ 24 for the renal cortex and 155 $\pm$ 13 for the renal medulla. After treatment with indomethacin, these values did not change significantly being 310 $\pm$ 18 for the renal cortex and 161 $\pm$ 12 for the renal medulla. Thus none of the treatments employed caused differential changes between renal cortex and medulla.

It is generally agreed that administration of DA can cause a decrease in the peripheral vascular resistance of many animals (although pretreatment with α-adrenoceptor blocking agents is sometimes necessary). The principle mechanism of the depressor action of DA in the rat is thought to be via activation of specific DA receptors and of β-adrenoceptors but not 5-hydroxytryptaminergic receptors, cholinergic receptors, histaminergic receptors nor by modification of the release of noradrenaline (Chapman et al 1980a).

Nevertheless, DA might act by stimulating the release of other vasodepressor agents such as endogenous prostaglandins. To investigate this hypothesis, the effect of indomethacin (a potent inhibitor of prostaglandin synthesis and release) on the vasodepressor and renal vasodilating actions of DA in the anaesthetized phenoxybenzamine- and propranolol-treated rat was in-

vestigated. If the responses to specific DA receptors were mediated by prostaglandins, then inhibition of prostaglandin synthesis and release would be expected to alter the vascular response to DA.

Arachidonic acid produced a decrease in the blood pressure of these rats and it is well established that this depressor response is caused by prostaglandins which are rapidly synthesized from the injected arachidonate. After indomethacin treatment there was a significant reduction in the response to arachidonic acid. This suggests that the dose of indomethacin used in the course of the experiments was sufficient to significantly reduce the production and release of prostaglandins in the rat. The indomethacin did not completely block the response to arachidonic acid. This is in agreement with Chevillard et al (1978), who suggested that the arachidonate itself may have slight vasodilator properties in its own right. Furthermore, there are many published results which show that this dose of indomethacin is more than sufficient to greatly inhibit the endogenous synthesis and release of prostaglandins (see review, Flowers 1974).

In this study, indomethacin slightly, but not significantly, reduced the depressor response to DA in phenoxybenzamine treated rats; had no effect on the depressor response to DA in phenoxybenzamine- and propranolol-treated rats; and did not inhibit the depressor response to isoprenaline in phenoxybenzamine-treated rats. These results indicate that prostaglandins are not involved in those responses which are mediated by β-adrenoceptors nor specific dopamine receptors.

Our results are not in agreement with those of Chevillard et al (1978) who found that about 50% of the depressor response to DA could be abolished by indomethacin. It is difficult to understand why such a difference should be found, since our results for the changes in MABP produced by DA injections were similar. However, different workers sometimes do obtain conflicting results when studying prostaglandins, and Tobian (1976) has suggested that these discrepancies may arise from differences in surgical technique.

Intra-arterial infusion of DA produced a decrease in the renal vascular resistance of phenoxybenzamine- and propranolol-treated rats, but indomethacin treatment had no significant effect on this phenomenon and it is therefore unlikely that prostaglandins contribute to the increase in renal blood flow and decrease in resistance to blood flow produced by DA. These observations are in agreement with those of Dressler, et al (1975), and Pendleton & Woodward (1976), who found that indomethacin treatment did not alter the renal response to DA in the dog, and Davila et al (1980) who found that indomethacin administration did not alter sodium excretion in rabbits; but differ (as discussed above) from those of Chevillard et al (1978).

In summary, the vasodilator and depressor actions of dopamine in the rat, (i.e. those mediated by β-adrenoceptors and by dopaminergic receptors) are not dependent on prostaglandin biosynthesis.

March 19, 1980

REFERENCES

Aukland, K. (1976) Int. Rev. Physiol. 11: 23–79

Chapman, B. J., Horn, N. M., Munday, K. A., Robertson, M. J. (1980a) J. Physiol. 298:437–452

Chapman, B. J., Lush, S. E., Munday, D. A. (1980) J. Physiol. 302:7–8P

Chevillard, C., Mathieu, M. N., Barjon, P. (1978) J. Pharm. Pharmacol. 30:329–330

Colquhoun, D. (1971) Lectures in Biostatistics, Oxford, Clarendon, p. 30

Davila, T., Davila, D., Oliw, E. Anggård, E. (1980) Acta Pharmacol. Toxicol. 46:57–61

Dressler, W. E., Rossi, G. V., Orzechowski, R. F. (1975) J. Pharm. Pharmacol. 27:203–204

Dunham, E. W., Zimmerman, B. G. (1970) Am. J. Physiol. 219:1279–1285

Eble, J. N. (1964) J. Pharmacol. Exp. Ther. 145:64–70

Flowers, R. J. (1974) Pharmacol. Rev. 26: 33–65

Goldberg, L. I. (1972) Pharmacol. Rev. 24:1–29

Gross, J. B., Bartter, F. C. (1973) Am. J. Physiol. 225: 218–224

Haining, J. L., Turner, M. D. (1966) J. Appl. Physiol. 21:1705–1708

Horton, E. W. (1974) in: Goodwin, T. W. (ed) MTP Int. Rev. Sci. Biochemistry of lipids, Biochemistry series 1, 4: pp 234–240

Pendleton, R. G., Woodward, P. W. (1976) Arch. Int. Pharmacodyn. Ther. 221:250–260

Tobian, L. (1976) in: Thurau, K. (ed) MTP Int. Rev. Physiol. Kidney and urinary tract physiology Vol II: pp 81–94