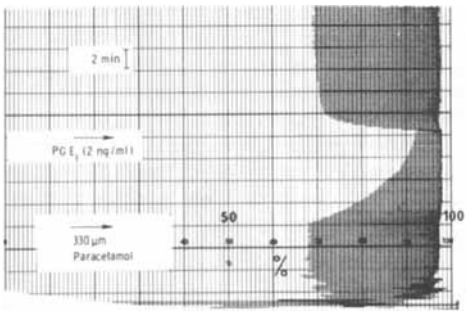

FIG. 2. Guinea-pig ileum myenteric plexus-longitudinal muscle preparation; negation of paracetamol-induced inhibition by PGE_1.

via opiate receptors. We have examined paracetamol and aspirin on other opiate sensitive tissue—the field stimulated mouse vas deferens preparation. At concentrations ranging from 10 μM–1 mM, paracetamol and aspirin were devoid of any inhibitory activity. In contrast, the tissue was sensitive to leu-enkephalin (IC50–15 nM). Furthermore, the inhibitory effect of paracetamol on the MPLM was not negated by the opiate antagonist naloxone at concentrations (10^{-7}–10^{-6} M) which totally antagonized the effect of leu-enkephalin.

Thus we have demonstrated that paracetamol depresses electrically evoked contractions of the MPLM. This effect is negated by PGE_1 which suggests the involvement of prostaglandins in this response.

Paracetamol has an IC50 of 95·2 μM on the MPLM. Flower & Vane (1974) noted that paracetamol inhibited prostaglandin synthesis in the rabbit brain at similar concentrations (IC50 92 μM). Bennett et al (1975) have suggested that prostaglandin synthetase might exist in two pools in the guinea-pig ileum—an extra-neuronal pool, which could be fairly sensitive to low concentrations of indomethacin, and a 'neuronal pool', which is only inhibited by high concentrations of indomethacin. Flower & Vane (1974) found indomethacin to be more potent against synthetase from dog spleen than from rabbit brain, thus demonstrating its low potency in a neuronal system. Conversely, paracetamol was several times more potent in the brain system than in the spleen. This may indicate that paracetamol is relatively specific for the neuronal synthetase system. This would explain why the rabbit brain (IC50 92 μM) and the MPLM (IC50 95·2 μM) are more sensitive to lower concentrations of paracetamol.

Since the concentrations of paracetamol found to be effective in this study are similar to plasma concentration found in man (Prescott et al 1968) it is likely that some inhibition of neuronal PG synthetase occurs during clinical use of this drug.

REFERENCES

Bennett, A., Eley, K. G., Stockley, H. L. (1975) Prostaglandins 9: 377–384

Ehrenpreis, S., Greenberg, J., Belman, S. (1973) Nature New Biology 245: 280–282

Flower, R. J., Vane, J. R. (1974) in: Robinson, H. J., Vane, J. R. (eds) Prostaglandin Synthetase Inhibitors. Raven Press New York, pp 978

Paton, W. D. M., Zar, M. A. (1968) J. Physiol. (London) 194: 13–33

Prescott, L. F., Sansur, M., Levin, W., Conney, A. H. (1968) Clin. Pharm. Ther. 9: 605–614

Shaw, J. S., Turnbull, M. J. (1978) Eur. J. Pharmacol. 49: 313–317

Sokunbi, Y. O. O. (1979) Br. J. Pharmacol. 67: 464P

J. Pharm. Pharmacol. 1981, 33: 189–191
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[^{3}H]Prazosin and [^{3}H]clonidine binding to α-adrenoceptors in membranes prepared from regions of rat kidney

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[^{3}H]Clonidine is known to bind with high affinity to membranes prepared from guinea-pig renal cortex, while the renal medulla and papilla exhibit much lower levels of specific binding (Summers 1980). Drug displacement studies have shown that the binding site has pharmacological characteristics similar to the α_2-adrenoceptor (Jarrott et al 1979). In contrast, negligible specific binding is observed with [^{3}H]prazosin, a ligand which selectively labels α_1-adrenoceptors (Greengrass & Bremner 1979). In this study, the binding characteristics of [^{3}H]clonidine and [^{3}H]prazosin have been investigated in membranes prepared from rat renal cortex and medulla.

Male Sprague-Dawley rats (150–225 g) were decapitated, and the kidneys rapidly removed and placed on ice. Longitudinal sections were made with a razor blade and the cortex and medulla (including papilla) separated by dissection. Membranes were prepared from these areas as previously described (Summers 1980). One ml aliquots of the final membrane preparation, containing 20 mg ml^{-1} of tissue, were incubated with an equal volume of Tris buffer (50 mM, pH 7·6

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Table 1. Displacement of [^{3}H]clonidine and [^{3}H]prazosin binding from membranes prepared from the rat renal cortex and medulla by α-adrenoceptor agonists and antagonists. Displacement curves were obtained using a concentration range between 0·1 nM and 10 μM of displacing drug and ≈1nM [^{3}H]clonidine or ≈0·5 nM [^{3}H]prazosin. The IC50 and slope of the log-logit plot (nH) were calculated using an iterative curve fitting program (see Methods) and the Ki (apparent inhibition constant) calculated using the Cheng & Prusoff equation (1973). Mean values ± s.e.m. were obtained from three experiments conducted in duplicate.

	[^{3}H]Clonidine binding				[^{3}H]Prazosin binding	
	Cortex		Medulla		Cortex	
Displacing drug	Ki nM	nH	Ki nM	nH	Ki nM	nH
(−)-Noradrenaline	7·2 ± 0·93	0·63 ± 0·07	7·3 ± 0·42	0·83 ± 0·03	1525 ± 293	0·47 ± 0·06
(+)-Noradrenaline	68·2 ± 6·8	0·68 ± 0·04	86 ± 6	0·78 ± 0·02	>10,000	—
Oxymetazoline	8·9 ± 1·8	0·57 ± 0·03	1·5 ± 0·1	0·65 ± 0·04	66 ± 5	0·59 ± 0·07
Yohimbine	133 ± 31	0·72 ± 0·01	100 ± 19	0·71 ± 0·02	391 ± 29	1·07 ± 0·04
Methoxamine	392 ± 45	0·61 ± 0·10	1122 ± 117	0·71 ± 0·11	9706 ± 967	0·62 ± 0·08
Prazosin	695 ± 123	0·55 ± 0·11	3540 ± 1092	0·49 ± 0·03	—	—
Clonidine	—	—	—	—	449 ± 46	0·73 ± 0·06
WB 4101*	—	—	—	—	1·43 ± 0·29	0·68 ± 0·36

* 2-(*N*-[2,6-dimethoxyphenyloxyethyl])-aminomethyl-1,4-benzodioxane.

at 25 °C) containing increasing amounts of labelled radioligand ([^{3}H]prazosin: Specific activity 33 Ci mmol^{-1}: final concentration ≈0·05–4 nM: [^{3}H]clonidine Specific activity 22 Ci mmol^{-1}: final concentration ~0·2–10 nM). At each concentration of radioligand, specific binding was determined using phentolamine (10 μM). With both [^{3}H]prazosin and [^{3}H]clonidine, specific binding represented from 90% (at low concentrations) to 50% (at high concentrations) of the total binding observed. At the end of the incubation, bound and free radioligand were separated by filtration using Whatman GF/B glass filters (U'Prichard et al 1977).

In drug displacement studies [^{3}H]prazosin (~0·5 nM) or [^{3}H]clonidine (~1 nM) were incubated with increasing amounts of displacing drug. As in the saturation studies non-specific binding was defined by 10 μM phentolamine. The concentration of drug displacing 50% of specific binding (IC50) was calculated using an iterative curve fitting computer program utilizing the equation

$$\% \text{ radioligand displaced} = \frac{100.[D]^P}{[D]^P + [IC50]^P}$$

where [D] is concentration of displacing drug and P is numerically equal to the gradient of the log-logit plot (Hill coefficient). From this IC50 the Ki was calculated using the method of Cheng & Prusoff (1973).

In the rat kidney, in contrast to the guinea-pig kidney, [^{3}H]clonidine was found to bind with high affinity to sites located in both the renal cortex and medulla. The concentration of sites in the cortex was 5·7 ± 0·9 p mol g^{-1} wet weight of tissue and in the medulla 3·4 ± 1·0 p mol g^{-1} wet weight of tissue. There were significant differences in the apparent dissociation constant (Kd) obtained from the two areas. In the cortex the Kd was 6·0 ± 1·0 nM (n = 3), similar to that found in the guinea-pig renal cortex (Summers 1980). In the medulla the Kd was 2·3 ± 0·6 nM (n = 3), similar to the value obtained using [^{3}H]clonidine in the guinea-pig spleen (McPherson & Summers 1980). In both cases the Scatchard plots were linear indicating binding to a single population of sites. The Hill coefficients, were not significantly different from unity, indicating a lack of co-operativity in binding.

In contrast to the more general distribution of sites labelled with [^{3}H]clonidine, [^{3}H]prazosin binding sites were highly localized to the renal cortex. The concentration of binding sites was 4·6 ± 0·6 pmol g^{-1} wet weight in the cortex while in the medulla the concentration was much less, 1·5 ± 0·9 pmol g^{-1} wet weight. The Kd's obtained in membranes prepared from both areas were similar and showed the binding to be of high affinity. In the cortex the Kd was 0·12 ± 0·01 nM and in the medulla 0·13 ± 0·04 nM (n = 3). As with [^{3}H]clonidine binding, Scatchard and Hill analysis indicated only a single population of sites with no co-operativity.

Both [^{3}H]clonidine and [^{3}H]prazosin binding were displaced by drugs known to act on α-adrenoceptors. For [^{3}H]clonidine in the renal cortex the rank order of potency was (—)-noradrenaline = oxymetazoline > (+)-noradrenaline > yohimbine > methoxamine > prazosin (Table 1). A similar rank order of potency was obtained for the same drugs in displacing [^{3}H]clonidine binding in the renal medulla (see Table 1).

The rank order of potency for displacing [^{3}H]prazosin binding in the renal cortex was WB 4101 > oxymetazoline > yohimbine > clonidine > (—)-noradrenaline > methoxamine > (+)-noradrenaline (Table 1).

The Hill coefficients obtained from the displacement curves (see Methods) are shown in Table 1. Values are given for drugs displacing [^{3}H]clonidine binding in both the renal cortex and medulla and for [^{3}H]prazosin binding in the cortex. All values were much less than unity except for yohimbine displacing [^{3}H]prazasin binding.

The results of the present study demonstrate a marked species difference in the regional distribution of [^{3}H]prazosin and [^{3}H]clonidine binding between rat and

guinea-pig kidney. In the rat [^{3}H]clonidine binding is present to a similar extent in both the cortex and medulla. The levels are somewhat higher than those reported in membranes from whole kidney (U'Prichard & Snyder 1979). The concentration of sites in the renal cortex of the rat is however about 1/3 of that of the guinea pig (Summers 1980) but the Kd is similar. In the membranes prepared from rat renal medulla there was a similar concentration of sites to that observed in the renal cortex. The major difference in the [^{3}H]clonidine binding observed in the two areas of the rat kidney is the difference in the apparent dissociation constant. In the rat renal medulla the binding was of higher affinity (Kd = 2·3 nM) than either rat or guinea-pig cortex (Kd 6·0 and 8·5 nM respectively). This higher affinity binding to membranes from the medulla is similar to that observed in the rat cerebral cortex (U'Prichard et al 1979; Jarrott et al 1979) guinea-pig spleen (McPherson & Summers 1980) and guinea-pig ileum (Tanaka & Starke 1979).

The characteristics of [^{3}H]prazosin binding have been described in rat brain (Hornung et al 1979; Greengrass & Bremner 1979) and lung (Barnes et al 1979). In these tissues prazosin binds with high affinity (Kd = 0·1–0·6 nM) to a site with characteristics suggesting binding to an α_1-adrenoceptor. The Kd obtained in the present study (Kd $\approx$ 0·1 nM) is similar to that observed in these other tissues. The highly localized binding of [^{3}H]-prazosin in the renal cortex membranes resembled that obtained with [^{3}H]clonidine in the guinea-pig renal cortex (Summers 1980).

Drug displacement studies in the rat kidney revealed that both [^{3}H]clonidine and [^{3}H]prazosin binding was displaced by drugs known to act on α-adrenoceptors. In addition in all areas, with the respective ligands, the (−)-isomer of noradrenaline was more potent in displacing binding than the (+)-isomer, indicating the binding site is stereo-selective as would be required for an α-adrenoceptor (Patil et al 1974). However, the slope of the log-logit plot calculated from the drug-displacement studies showed a marked difference from one drug to the next, only for yohimbine displacing [^{3}H]prazosin binding was the gradient near unity. The exact reason for the variation is unknown, particularly since it appears unrelated to the pharmacological nature of the radioligand or displacer i.e. agonist displacing an agonist or antagonists displacing an antagonist. However, similar variations in the gradient are observed when the results of experiments using the same displacing agents against [^{3}H]clonidine binding in the ileum (Tanaka & Starke 1979) and with [^{3}H]clonidine and [^{3}H]prazosin binding in rat brain (Hornung et al 1979), are replotted as log-logit plots. In membranes prepared from guinea-pig ileum the gradients of the log-logit plots of displacement of [^{3}H]clonidine binding were less than unity for both clonidine and prazosin. In membranes from rat brain prazosin and yohimbine displaced [^{3}H]prazosin binding with gradients of unity but oxymetazoline and clonidine produced gradients of 0·55 and 0·61 respectively. The gradients of the log-logit plots for [^{3}H]clonidine binding were near unity for displacement by clonidine and oxymetazoline but less than unity for yohimbine (0·8) and prazosin (0·77). While these differences may indicate that there is negative co-operatively involved in displacement of ligands by some drugs acting on α-adrenoceptors, the low values for the Hill coefficient could also indicate that the ligands and/or the displacing drugs are binding to more than one site. Certainly in kinetic experiments [^{3}H]clonidine is known to bind to two distinct sites (U'Prichard et al 1979) and the Ki values reported here in Table 1 indicate that for many of the compounds there will be appreciable binding to sites not occupied by the particular ligand used, which for the less selective drugs would have the effect of decreasing the gradient of the displacement curve.

In conclusion, the results of this study indicate that both [^{3}H]clonidine and [^{3}H]prazosin bind with high affinity to sites in rat kidney. [^{3}H]Clonidine binding is found in both renal cortex and medulla whereas [^{3}H]prazosin binding is localized to the cortex. These results are in contrast to those obtained in the guinea-pig where [^{3}H]clonidine binding is localized to the cortex and no significant [^{3}H] prazosin binding is observed.

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REFERENCES

Barnes, P., Karlinger, J., Hamilton, C., Dollery, C. (1979) Life Sci. 25: 1207–1214

Cheng, Y., Prusoff, W. H. (1973) Biochem. Pharmacol. 22: 3099–3108

Greengrass, P., Bremner, R. (1979) Eur. J. Pharmacol. 55: 323–326

Hornung, R., Presek, P., Glossmann, H. (1979) Naunyn Schmiedeberg's Arch. Pharmacol. 308: 223–230

Jarrott, B., Louis, W. J., Summers, R. J. (1979) Br. J. Pharmacol. 65: 663–670

McPherson, G. A., Summers, R. J. (1980) Clin. Exp. Physiol. Pharmacol. in the press

Patil, P. N., Miller, D. D., Trendelenburg, H. (1974) Pharmacol. Rev. 26: 323–392

Summers, R. J. (1980) Br. J. Pharmacol. 71: 57–64

Tanaka, T., Starke, K. (1979) Naunyn Schmiedeberg's Arch. Pharmacol. 309: 207–215

U'Prichard, D. C., Greenberg, D. A., Snyder, S. H. (1977) Mol. Pharmacol. 13: 454–473

U'Prichard, D. C., Bechtel, W. D., Rouot, B. M., Snyder, S. H. (1979) Mol. Pharmacol. 16: 47–60

U'Prichard, D. C., Snyder, S. H. (1979) Life Sci. 29: 79–88