J. Pharm. Pharmac., 1975, **27**, 262–267 *Received July* 12, 1974

The effect of acute bilateral adrenalectomy on vasopressor responses to catecholamines in dogs

C. T. CHOPDE, D. M. BRAHMANKAR, R. V. SHEOREY, A. G. UDHOJI AND A. K. DORLE

Department of Pharmaceutical Sciences, University Campus, Amaraoti Road, Nagpur-440010 *M.S. (India)*

The effect of acute bilateral adrenalectomy on the pressor responses to adrenaline, noradrenaline and isoprenaline was studied in anaesthetized dogs. The responses to all the three catecholamines were reduced by adrenalectomy. Treatment with cortisone, cyclic AMP partially restored the responsiveness. Desoxycorticosterone, aldosterone, hydrocortisone, phenylbutazone or infusion of either saline and noradrenaline failed to improve the impaired pressor responses seen in adrenalectomized dogs. Treatment with corticosterone alone, combined administration of aldosterone and hydrocortisone or cortisone followed by cyclic 3′,5′-AMP also restored catecholamine responses almost to normal. The pressor responses to catecholamines in dogs were also reduced by metyrapone-induced cortical insufficiency. Administration of corticosterone, cortisone or cyclic AMP slightly improved these responses; the recovery was not, however, as effective as that noted in the adrenalectomized condition.

Reports on the effect of adrenalectomy on pressor responses to catecholamines are conflicting. While Salmoiraghi & McCubbin (1954); Brown & Remington (1955); Remington, Collings & others (1941) failed to observe any effect in dogs, other workers (Fritz & Levine, 1951; Drew & Leach, 1970; Cartoni & Carpi, 1968) have observed that the cardiovascular sensitivity to noradrenaline is significantly reduced by adrenalectomy in rats. It was, therefore, considered necessary to re-examine this aspect in dogs.

MATERIALS AND METHODS

Dogs of either sex between 10–15 kg were anaesthetized with sodium phenobarbitone (150 mg kg^{-1}, i.p.). The trachea was cannulated and total bilateral adrenalectomy was performed using the dorsal approach. Dogs were not maintained on saline as such treatment does not assist in producing a true adrenalectomized condition (Brodie, Davies & others, 1966). After adrenalectomy a rest period of 3 h was allowed. The blood pressure was recorded on smoked paper using a mercury manometer inserted into the carotid artery. The femoral vein was cannulated for intravenous injections. After recording the control initial responses to varying doses (0·2–1 μg^{-1}) of adrenaline (BDH), noradrenaline (Fluka) and isoprenaline (K. & K. Labs) (all expressed as salts) the effect of (1) mineral corticoids (0·5–20 mg kg^{-1}) (aldosterone; desoxycorticosterone acetate, Merck), (2) glucocorticoids (1–20 mg kg^{-1}) (hydrocortisone; cortisone acetate (Merck), (3) corticoids of mixed activity (0·5–10 mg kg^{-1}) (corticosterone, Organon); (4) mixture of aldosterone (20 mg kg^{-1}) and hydrocortisone (20 mg kg^{-1}), (5) cyclic 3′, 5′-AMP (0·01–1 mg kg^{-1},

Sigma); (6) cortisone (20 mg kg^{-1}) followed by cyclic 3′,5′-AMP (1 mg kg^{-1}); (7) phenylbutazone 20–100 mg kg^{-1}, Geigy); (8) saline infusion (0·1 ml kg^{-1} min^{-1}) and (9) noradrenaline infusion (200–250 ng kg^{-1} min^{-1}) over periods of up to 30 min was studied on separate groups (10 dogs in each) of acutely adrenalectomized dogs. Responses to adrenaline, noradrenaline and isoprenaline were recorded at 30 min intervals over 2 h after commencement of blood pressure recordings.

Similar experiments were also made in sham-operated dogs where incision was made but the adrenal glands were not disturbed.

To the other groups of dogs (10 animals in each) metyrapone (20 mg kg^{-1}, i.v. daily—Ciba of India) was given for eight days. The animals were then prepared for blood pressure measurements and the effect of (1) corticosterone (5 mg kg^{-1}), (2) cortisone acetate (20 mg kg^{-1}), (3) cyclic 3′,5′-AMP (1 mg kg^{-1}) and (4) cortisone followed by cyclic 3′,5′-AMP on blood pressure responses to the three catecholamines was studied at 30 min intervals over 2 h.

All corticosteroids and phenylbutazone were administered by intramuscular injections whilst the other drugs were injected intravenously. Apart from the corticosteroids, all drugs were injected in 0·9% sodium chloride solution and doses calculated as the salt except catecholamines which were made up from stock solutions (1 mg ml^{-1} calculated as the base) stored in 0·01N hydrochloric acid. Corticosteroid suspensions were made as 100 mg ml^{-1} in 2% w/v carboxymethylcellulose.

RESULTS

Tables 1 and 2 show that the blood pressure responses to adrenaline, noradrenaline and isoprenaline were significantly reduced in adrenalectomized dogs compared to the sham operated controls. The initial resting blood pressure in adrenalectomized dogs was also lower than in the controls. Cortisone (20 mg kg^{-1}) or cyclic 3′,5′-AMP (1 mg kg^{-1}) increased the responsiveness to catecholamines in adrenalectomized dogs (Table 1). Treatment with corticosterone (5 mg kg^{-1}) and combined administration of aldosterone (20 mg kg^{-1}) and hydrocortisone (20 mg $kg)^{-1}$ (Table 2) or cortisone (20 mg kg^{-1}) followed by cyclic 3′,5′-AMP (1 mg kg^{-1}, Table 1) also partially restored the catecholamine responses. The restoration was seen to be maximal at 2 h after the corticosteroid administration. After administration of 1 mg kg^{-1} or less of corticosterone the blood pressure responses to catecholamines were much smaller than that observed in sham operated dogs; 2·5 mg kg^{-1} of corticosterone produced an unreliable extent of sensitivity restoration, however, when the dose was increased to 5 mg kg^{-1} the responsiveness to catecholamines was maximally restored almost to the levels observed in sham preparations. Corticosterone produced no further significant increase in the responsiveness. Although the infusion of noradrenaline increased the basal blood pressure by about 15 mm, the responsiveness to injected catecholamines was not restored.

Aldosterone (0·5–20 mg kg^{-1}), desoxycorticosterone (0·5–20 mg kg^{-1}), hydrocortisone (1–20 mg kg^{-1}), phenylbutazone (20–100 mg kg^{-1}) and saline infusion (0·1 ml kg^{-1} min^{-1}) were unable to restore the blood pressure responses to catecholmines in adrenalectomized dogs.

None of the corticosteroids had any effect on catecholamine responses in sham operated animals.

The blood pressure responses to catecholamines were also reduced in metyrapone-

Table 1. *Influence of cyclic 3',5'-AMP and cortisone on catecholamine-induced changes in mean arterial blood pressure (mm Hg) in adrenalectomized dogs.* The magnitude of pressor and depressor responses were measured as increases or decreases respectively from basal levels.

Drug	Dose $\mu g\ kg^{-1}$	Sham operated control (140 ± 4·6)	After adrenalectomy (90 ± 1·7)*	Cyclic AMP 1 mg kg^{-1} (90 ± 2·1)	Cortisone 20 mg kg^{-1} (100 ± 3·2)*	Cortisone followed by cyclic AMP (100 ± 4·6)*
Adrenaline	0·2	30·0 ± 0·8	12·8 ± 0·6	20·2 ± 0·5*	21·4 ± 0·8	26·3 ± 0·7
	0·4	38·6 ± 1·0	18·0 ± 0·8	24·4 ± 0·6*	27·5 ± 1·0	34·6 ± 0·5
	0·6	50·0 ± 0·9	27·1 ± 0·8	32·2 ± 0·8*	35·2 ± 0·8	43·1 ± 1·0
	0·8	59·2 ± 0·8	29·1 ± 0·5	35·2 ± 1·0*	40·0 ± 1·0	50·4 ± 0·6
	1·0	65·0 ± 1·0	31·3 ± 0·6	43·4 ± 0·4*	49·3 ± 0·6	56·2 ± 0·8
Noradrenaline	0·2	42·8 ± 1·5	17·0 ± 0·4	26·3 ± 0·2*	24·0 ± 0·5	34·3 ± 0·6
	0·4	55·2 ± 1·0	25·2 ± 0·6	31·7 ± 0·7*	32·2 ± 0·9	41·4 ± 0·8
	0·6	65·0 ± 0·7	32·0 ± 0·6	40·2 ± 0·5*	46·0 ± 1·0	52·3 ± 0·5
	0·8	73·2 ± 0·5	36·0 ± 0·9	46·3 ± 1·0*	55·2 ± 0·4	65·2 ± 0·6
	1·0	84·0 ± 0·8	38·0 ± 1·0	50·4 ± 0·7*	60·2 ± 0·6	69·4 ± 0·6
Isoprenaline	0·2	34·0 ± 1·4	10·5 ± 0·5	15·3 ± 0·7*	20·0 ± 0·8	26·3 ± 0·3
	0·4	45·0 ± 1·5	13·0 ± 0·9	18·2 ± 0·4*	28·5 ± 1·0	33·2 ± 0·5
	0·6	58·0 ± 0·9	15·2 ± 0·6	15·7 ± 1·0	33·2 ± 0·5	36·4 ± 0·2
	0·8	70·0 ± 1·0	18·0 ± 1·0	18·0 ± 0·3	39·2 ± 1·0	40·3 ± 0·4
	1·0	76·0 ± 0·7	25·0 ± 1·0	25·0 ± 0·6	47·0 ± 0·9	47·0 ± 0·3

Figures in the parentheses represent the initial basal blood pressure level.
± Standard error of mean of five observations.
* Differences between the control and experimental values significant at ($P < 0·05$).

Table 2. *Influence of corticosterone and combined administration of aldosterone and hydrocortisone on catecholamine induced blood pressure responses in adrenalectomized dogs.* The magnitude of pressor and depressor responses were measured as increases or decreases respectively from basal levels.

Drug	Dose $\mu g\ kg^{-1}$	Sham operated controls (135 ± 3·5)	After adrenalectomy (90 ± 0·8)	Corticosterone 5 mg kg^{-1} (110 ± 2·9)	Aldosterone 20 mg kg^{-1} + hydrocortisone 20 mg kg^{-1} (105 ± 3·8)
Adrenaline	0·2	28·6 ± 0·5	11·8 ± 0·6	26·8 ± 0·4	26·0 ± 0·5
	0·4	35·7 ± 0·8	17·5 ± 0·4	33·2 ± 0·7	33·8 ± 0·7
	0·6	47·8 ± 1·0	26·1 ± 0·8	45·0 ± 0·5	44·3 ± 1·0
	0·8	58·2 ± 1·3	20·0 ± 0·5	54·1 ± 0·8	50·2 ± 0·6
	1·0	65·3 ± 0·8	31·0 ± 0·7	60·0 ± 0·5	57·4 ± 0·7
Noradrenaline	0·2	40·5 ± 0·5	15·4 ± 0·3	35·3 ± 0·4	34·0 ± 0·6
	0·4	51·2 ± 1·2	23·6 ± 0·7	42·0 ± 0·8	45·3 ± 0·7
	0·6	63·3 ± 0·6	32·0 ± 0·7	57·3 ± 0·6	51·3 ± 0·5
	0·8	74·0 ± 0·8	36·0 ± 0·9	67·7 ± 0·3	62·0 ± 0·6
	1·0	80·0 ± 1·0	37·8 ± 0·5	71·8 ± 0·4	69·0 ± 0·3
Isoprenaline	0·2	30·7 ± 0·8	10·5 ± 0·5	25·7 ± 0·3	24·2 ± 0·3
	0·4	41·0 ± 1·2	12·8 ± 0·8	36·2 ± 0·7	35·3 ± 0·2
	0·6	56·6 ± 0·5	15·5 ± 0·6	45·0 ± 0·4	44·2 ± 0·5
	0·8	67·3 ± 0·9	17·3 ± 1·0	50·0 ± 0·3	49·2 ± 0·2
	1·0	74·9 ± 0·8	24·8 ± 0·4	51·3 ± 0·4	50·8 ± 0·2

Figures in the parentheses represent the initial basal blood pressure level.
± Standard error of mean of five observations.
All the values were significant ($P < 0·05$).

induced cortical insufficiency in dogs. Administration of cortisone or corticosterone partially improved these responses; the recovery was, however, smaller in the metyrapone-treated dogs than in adrenalectomized anaimals. Cyclic 3′, 5′-AMP following cortisone caused further improvement in the pressor responses to catecholamines in metyrapone-treated dogs (Table 3).

Table 3. *Effect of cortisone and cyclic 3′,5′-AMP on changes in mean arterial blood pressure mm Hg induced by catecholamines in metyrapone-treated dogs.* The magnitude of pressor and depressor responses were measured as increases or decreases respectively from basal levels.

			Responses in metyrapone-treated dogs		
Drug	Dose $\mu g\ kg^{-1}$	Control (100 ± 3·8)	Cortisone 20 mg kg^{-1} (100 ± 3·1)	Cyclic AMP 1 mg kg^{-1} (80 ± 4·3)	Cortisone followed by cyclic AMP (100 ± 4·0)*
Adrenaline	0·2	16·0 ± 0·5	20·0 ± 0·7*	22·2 ± 0·4*	30·0 ± 0·4
	0·4	20·1 ± 0·7	25·7 ± 0·8*	27·0 ± 0·7*	38·4 ± 0·5
	0·6	27·2 ± 0·4	33·3 ± 0·4*	33·0 ± 0·6*	42·2 ± 0·8
	0·8	30·0 ± 0·8	34·2 ± 0·6*	36·2 ± 0·6*	45·3 ± 0·7
	1·0	32·5 ± 0·3	35·3 ± 0·8	39·2 ± 0·5*	50·3 ± 0·5
Noradrenaline	0·2	20·2 ± 0·6	25·3 ± 0·4*	27·2 ± 0·6*	33·3 ± 0·5
	0·4	28·0 ± 0·4	35·0 ± 0·3*	34·0 ± 0·5*	40·4 ± 0·3
	0·6	33·2 ± 0·7	40·0 ± 0·8*	39·3 ± 0·8*	47·2 ± 0·6
	0·8	38·5 ± 0·5	40·3 ± 0·5	44·0 ± 0·8*	50·3 ± 0·8
	1·0	40·4 ± 0·4	44·2 ± 1·0*	47·2 ± 0·7*	59·2 ± 0·7
Isoprenaline	0·2	15·0 ± 0·3	21·3 ± 0·5*	20·0 ± 0·3*	26·0 ± 0·3
	0·4	20·2 ± 0·2	25·2 ± 0·5*	25·3 ± 0·4	32·2 ± 0·4
	0·6	26·2 ± 0·4	30·2 ± 0·3*	30·7 ± 0·4	37·2 ± 0·6
	0·8	28·0 ± 0·3	34·0 ± 0·4*	30·2 ± 0·2	38·0 ± 0·4
	1·0	30·0 ± 0·3	35·0 ± 0·6*	30·0 ± 0·2	35·0 ± 0·3

Figures in the parentheses represent the initial basal blood pressure level.
± Standard error of mean of five observations.
* Differences between the control and experimental values were significant ($P < 0·05$).

DISCUSSION

The impaired pressor responses to noradrenaline in dogs have not been found by Salmoiraghi & McCubbin (1954), Brown & Remington (1955) or Remington & others (1941). Our findings of reduced pressor responses to the catecholamines in adrenalectomized dogs are in accordance with those of others in rats (Ramey & Goldstein, 1957; Bush, 1962, Cartoni & Carpi, 1968, Drew & Leach, 1970), and dogs (Ramey, Goldstein & Levine, 1951). It must be pointed out that the resting or basal blood pressure level also decreased as a result of adrenalectomy. The recovery of both the catecholamine and basal blood pressure after treatment with corticosterone but not after aldosterone, desoxycorticosterone and hydrocortisone, suggests that mineralcorticoids or glucocorticoids individually cannot counteract the loss of sensitivity to catecholamines in adrenalectomized dogs. The partial recovery seen with cortisone may be attributed to its glycogenic as well as its slight sodium retention property. Corticosterone, possessing mixed properties, is more effective. The striking effectiveness of the combination of aldosterone and hydrocortisone in

restoring the responses to control levels further emphasizes the requirement for both mineral and glucocorticoid properties. The inability of desoxycorticosterone and saline (Carpi & Cartoni, 1968) and hydrocortisone (Drew & Leach, 1970; Rosenfield, Sevey & Ohler, 1959), or the effectiveness of cortisone (Drew & Leach, 1970; Fritz & Levine, 1951) and corticosterone (Drew & Leach, 1971; Imms & Jones, 1967), in restoring the cardiovascular sensitivity during adrenocortical insufficiency in rats has already been commented upon. The loss of cardiovascular sensitivity to catecholamines in adrenocortical insufficiency can, therefore, be correlated with the overall imbalance in the ionic environment and consequent metabolic disturbances. The possibility that surgical shock alone might be responsible for the loss of sensitivity is excluded by the fact that administration of phenylbutazone failed to improve the pressor responsiveness (Goodman & Gilman, 1965; Krawczak, 1969). The ineffectiveness of noradrenaline infusion to restore the pressor responses to catecholamines in adrenalectomized dogs suggests that changes in background circulatory level of neurotransmitter could not be responsible for the changes following adrenalectomy as reported by Harkal, Sevy & others (1968).

Takabatake (1966) has observed that the elevation of cyclic 3′,5′-AMP levels cannot be induced by adrenaline in adrenalectomized animals. On the contrary Murad, Chi & others (1962) have reported the activation of adenylcyclase by adrenaline in tissue homogenates from adrenalectomized animals. In this study we could detect some improvement in the catecholamine responses in the adrenalectomized dogs after giving cyclic 3′,5′-AMP while the combined treatment of cortisone and cyclic AMP was more effective in increasing the vasopressor responsiveness to catecholamines. Since it has been reported that cyclic 3′,5′-AMP levels are reduced in conditions of cortical insufficiency (Brodie & others, 1966), these changes could account for the disturbances in metabolic reactions and ionic permeability. Cyclic AMP is well known for its regulatory action on carbohydrate and lipid metabolism through activation of phosphorylase (Kreb, Graves & Fisher, 1959; Posner, Stern & Kreb, 1962) and lipase (Robinson, Butcher & others, 1965; Lundholm, Mohme-Lundholm & Sydmer, 1966). It is further suggested that the action of corticoids is mediated through a membrane effect which facilitates access of catecholamines to the intracellularly located enzyme and that the effect of adrenalecomy is to inhibit the step between the release of adrenergic neurohormone and the formation of cyclic 3′,5′-AMP (Brodie & others, 1966).

In the present study metyrapone was also used to induce adrenocortical insufficiency. Metyrapone can promptly inhibit hydrocortisone production through inhibition of 11β-hydroxylation (Goodman & Gilman, 1970), the biosynthetic process being opposed at the level of 11-desoxycortisol which has practically no inhibitory influence on ACTH release (Dominguez & Samuel, 1962; Henke & Doe, 1967). The reduction in catecholamine responses in metyrapone-treated dogs further supports our findings that the reduction in pressor responses to catecholamines are the result of lowered levels of circulating corticoids.

REFERENCES

BRODIE, B. B., DAVIES, J. I., GYNIE, S., KRISHNA, H. & WEISS, B. (1966). *Pharmac. Rev.*, **18**, 273–289.

BROWN, F. K. & REMINGTON, J. W. (1955). *Am. J. Physiol.*, **182**, 279–284.

BUSH, I. E. (1962). *Pharmac. Rev.*, **14**, 317–445.

CARPI, A. & CARTONI, C. (1968). *Br. J. Pharmac.*, **34**, 259–266.

CARTONI, C. & CARPI, A. (1968). *Ann. Ist Super. Sanita*, **4**, 338–340.

DOMINGUEZ, O. V. & SAMUEL, L. T. (1963). *Endocrinology*, **73**, 304–309.

DREW, G. M. & LEACH, G. D. H. (1971). *Archs int. Pharmacodyn. Thér.*, **191**, 255–260.

DREW, G. M. & LEACH, G. D. H. (1970). *J. Pharm. Pharmac.*, **22**, 811–817.

FRITZ, I. & LEVINE, R. (1951). *Am. J. Physiol.*, **165**, 456–465.

GOODMAN, L. S. & GILMAN, A. (1965). In *The Pharmacological Basis of Therapeutics*, p 337, New York: MacMillan,

GOODMAN, L. S. & GILMAN, A. (1970). *Ibid.*, p 478.

HARKAL, C., SEVY, R. W., REIDENBERG, M. M. & FAUST, R. E. (1968). *J. Pharmac. exp. Ther.*, **106**, 292–299.

HENKE, W. K. & DOE, R. P. (1967). *J. clin. Endocr. Metab.*, **27**, 1565–1572.

IMMS, F. J. & JONES, M. T. (1967). *J. Endocr.*, **38**, XVII.

KRAWCZAK, J. (1969). *Acta. Physiol. Pol.*, **20**, 237–246.

KREB, E. H., GRAVES, D. J. & FISHER, E. H. (1959). *J. biol. Chem.*, **234**, 2867–2873.

LUNDHOLM, L. MOHME-LUNDHOLM, E. & SYDMER, N. (1966). *Pharmac. Rev.*, **18**, 255–272.

MURAD, F., CHI, Y. M., RALL, T. W. & SUTHERLAND, E. W. (1962). *J. biol. Chem.*, **237**, 1233–1238.

POSNER, J. B., STERN, R. & KREB, E. H. (1962). *Biochem. biophys. Res. Commun.*, **9**, 293–296.

RAMEY, E. R. & GOLDSTEIN, M. S. (1957). *Physiol. Rev.*, **37**, 155–195.

RAMEY, E. R., GOLDSTEIN, M. S. & LEVINE, R. (1951). *Am. J. Physiol.*, **165**, 450–455.

REMINGTON, J. W., COLLINGS, W. D., HAYS, H. W., PARKINS, W. M. & SWINGLE, W. W. (1941). *Ibid.*, **132**, 622–628.

ROBINSON, G. A., BUTCHER, R. W., OYE, I., MORGAN, H. E. & SUTHERLAND, E. W. (1965). *Mol. Pharmac.*, **1**, 168–177.

ROSENFELD, H., SEVY, R. W. & OHLER, E. A. (1959). *Proc. Soc. exp. Biol. Med.*, **100**, 800–802.

SALMOIRAGHI, G. C. & MCCUBBIN, J. W. (1954). *Circ. Res.*, **2**, 280–284.

TAKABATAKE, E. (1966). *Pharmac. Rev.*, **18**, 280.