Br. J. Pharmac. (1968), **34**, 259–266.

Effects of guanethidine on the blood pressure response to splanchnic nerve stimulation in the rat: role of the adrenal medulla

A. CARPI AND CARLA CARTONI

Department of Therapeutical Chemistry, Istituto Superiore di Sanità, Rome, Italy

1. Splanchnic nerve stimulation provokes a larger increase in blood pressure in intact rats than in rats previously demedullated by means of expression of the adrenals *in situ* and adrenalectomy followed by adrenocortical grafting.

2. Rats pretreated with guanethidine showed an attenuated albeit prolonged hypertensive response to splanchnic stimulation. Similar effects were exerted by guanethidine in rats demedullated by expression. In rats demedullated by grafting, smaller doses of guanethidine induced a more substantial depression of the blood pressure response without increasing its duration.

3. It is concluded that inhibition of the peripheral sympathetic endings induced by guanethidine was partially compensated by the enhancement of the cardiovascular effects of the mediators released by the adrenal medulla and that demedullation by expression did not completely eliminate chromaffin tissue.

The role of the adrenal medulla in the pressor response to splanchnic nerve stimulation and in the changes induced by different drugs on this response have previously been studied by comparing splanchnic nerve stimulation before and after acute ablation or vascular exclusion of the suprarenal glands (Nickerson & Goodman, 1947; De Vleeschhouwer, 1935). Thus it has been observed that acute adrenalectomy abolishes the pressor response to splanchnic stimulation which is still present in cats after administration of guanethidine; hence it has been concluded that the arterial hypertension, evoked by splanchnic stimulation in cats treated with guanethidine, is due to the release of mediators from the adrenal medulla (Abercrombie & Davies, 1963). A reliable quantitative evaluation of the adrenomedullary contribution to the pressor effects of splanchnic stimulation cannot, however, be made by this experimental procedure. In fact, acute adrenalectomy involves a variable and often severe degree of surgical trauma which may in itself affect the cardiovascular reactivity non-specifically. Moreover, numerous early side effects of guanethidine (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960) may obscure its principal pharmacological action, consisting of the inactivation of the sympathetic nerve terminals, which is still present some hours after the administration of the drug (Chang, Costa & Brodie, 1965). In the present work, therefore, the effects of guanethidine pretreatment on the pressor response to splanchnic stimulation were compared in intact and chronically adrenal demedullated rats. The results obtained in rats which had undergone adrenal demedulla-

tion by the widely used method of expression of the gland suggested the desirability of a further study on the effects of the less frequently used method of adrenal demedullation by cortical grafting (Skelton, 1959).

Methods

Male albino rats (Wistar-derived strain maintained at our Institute) were anaesthetized with ethylurethane (1 g/kg intraperitoneally). Carotid blood pressure was recorded by means of an electromanometer connected with a Grass polygraph. Under artificial respiration (miniature Starling pump; rate 36/min ; stroke/volume 6–7 ml.), a left thoracotomy was performed between the eighth and ninth rib and the splanchnic nerve was exposed in the costophrenic sinus by gentle dissection of the prevertebral muscles. The nerve was placed on a bipolar silver electrode ; for stimulation, rectangular pulses of 6 V and of 1 msec duration were applied for 40 sec. In each experiment four successive stimulations were performed at a frequency of 4, 8, 12 and 20 c/s.

Adrenal demedullation was performed in rats under ether anaesthesia by two methods: either the adrenals were exposed through a lumbar approach, the adrenal cortex was incised and the medulla extruded by gentle squeezing of the gland with forceps (adrenal demedullation by expression) or bilateral adrenalectomy was performed and a small piece of adrenal cortex grafted under the capsule of each kidney (adrenal demedullation by adrenalectomy with cortical grafting). The animals were treated with penicillin (10,000 u/day) for 4 days and used for the acute experiment 30–60 days later. At the end of the experiment the remaining adrenal cortex or graft in the demedullated rats was stained by the chromaffin reaction (Hillarp & Hökfelt, 1955) and examined macroscopically 24 hr later. The results were discarded if any chromic impregnation was found.

The response to splanchnic stimulation was assessed by measuring the maximal increase in arterial pressure and the duration of this rise—that is, the time (in sec) between the termination of stimulation and the point at which blood pressure had fallen mid-way between the maximal and the prestimulation levels (half-life of the response). A covariance analysis (Snedecor, 1956) was performed on the results.

Results

Splanchnic nerve stimulation in the intact and adrenal demedullated rats

After preparing the splanchnic nerve for stimulation, the arterial pressure was 60.5 ± 4.4 mm Hg (mean $\pm$ S.E.) in intact rats, 59.0 ± 5.9 in adrenal demedullated rats by expression and 55.7 ± 5.0 in rats demedullated with cortical grafting ; the differences between these values were not significant ($P > 0.05$). In normal rats and in those adrenal demedullated by both methods, splanchnic nerve stimulation provoked a rise in the arterial pressure ; in some cases the rise was preceded, especially at the lower rates of stimulation, by a small and transient fall in arterial pressure (Fig. 1, B and C). An increase in stimulation frequency provoked a more substantial rise in arterial pressure (Fig. 1, A, B, and C) ; a significant linear regression was found by plotting the average blood pressure response to the different rates of stimulation against the log of the stimulation frequency (Fig. 3 and Table 1). In normal rats the pressor response was already maximal at 12 c/s ; therefore the regression calculated on the data obtained with the four rates of stimulation showed

a significant deviation from linearity ($F=7.46$; $P<0.005$). This deviation was not significant if the regression was calculated on data obtained with 4, 8 and 12 c/s. In adrenal demedullated rats no significant deviation from linearity was found in the curves calculated on data obtained with the four rates of stimulation. Comparison of the frequency-response curve was therefore based on data obtained in untreated intact rats with the first three rates of stimulation, and on data obtained in all other experimental groups with four rates of stimulation. The regression coefficient of the frequency-response curve obtained in fifteen normal rats was significantly higher than that obtained in sixteen rats demedullated by expression ($F=24.5$; $P<0.001$) and that obtained in seven rats demedullated by adrenalectomy with cortical grafting ($F=28.9$; $P<0.001$). No significant difference in the slope ($F=0.045$; $P>0.05$) and in the elevation ($F=2.0$; $P>0.05$) between the frequency-response curve of the two groups of demedullated rats was noted. A significant regression was found between the half-life of the blood pressure response and the log of the stimulation frequency only in untreated intact and demedullated rats by expression (Fig. 3 ; Table 1).

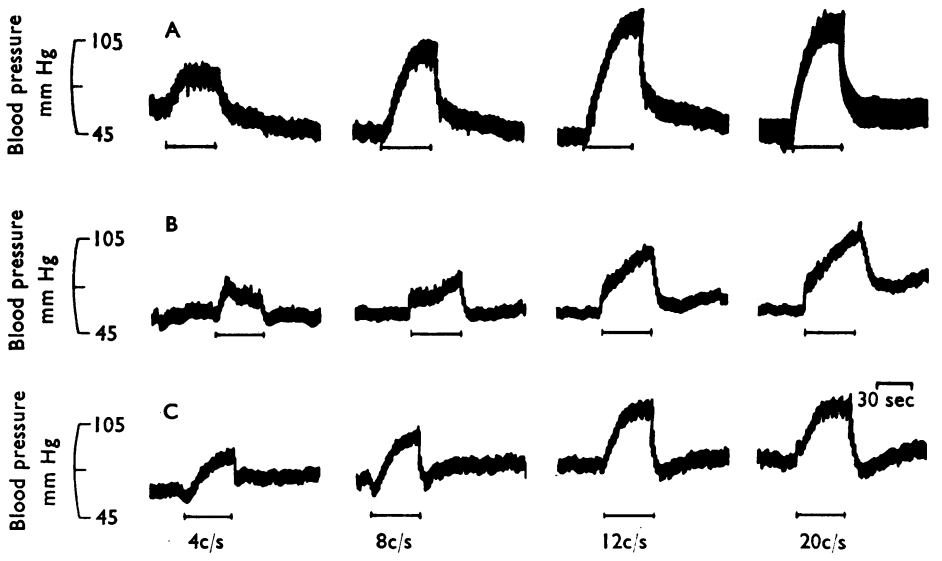

FIG. 1. Effects of different rates of stimulation (4, 8, 12 and 20 c/s) on the blood pressure response of (A) intact rats and rats demedullated by (B) expression and (C) grafting. The horizontal bar indicates the time and the duration of the stimulation.

TABLE 1. *Regression of the blood pressure response (mm Hg) and of the its half-life (sec) on the log of the stimulation frequency in untreated and guanethidine pretreated intact and demedullated rats*

Group	Guanethidine (mg/kg s.c.)	No. of experiments	Blood pressure response $\bar{y}$	Blood pressure response $b\pm s_b$	Blood pressure response P	Half-life $\bar{y}$	Half-life $b\pm s_b$	Half-life P
Intact	—	15	34·20	79·76±6·9	<0·001	5·00	8·26±2·3	<0·001
	20	9	30·94	57·49±6·7	<0·001	13·86	25·52±4·9	<0·001
Adrenal demedullated	—	16	24·04	35·06±5·2	<0·001	4·84	7·81±2·7	<0·01
by expression	20	6	18·96	37·49±4·7	<0·001	24·50	64·25±9·1	<0·001
Adrenal demedullated	—	7	27·11	33·09±4·8	<0·001	3·79	−0·57±3·5	>0·05
by grafting	7	8	12·62	17·44±3·5	<0·001	3·00	3·21±2·1	>0·05

The value of $\bar{x}$ is 0·8614 for the group of untreated intact rats and 0·9713 for all other groups. P indicates the significance of the regression coefficient b.

Effects of guanethidine pretreatment

Before splanchnic nerve stimulation, the arterial pressure of intact rats pretreated with guanethidine (20 mg/kg subcutaneously 16–20 hr before the experiment) was 56.5 ± 3.8 mm Hg ; this figure was not significantly different from that of untreated controls ($P>0.05$). In the intact rats, guanethidine pretreatment reduced the absolute values of the hypertensive response to splanchnic nerve stimulation, as confirmed by the significant reduction of the slope of the frequency-response curve

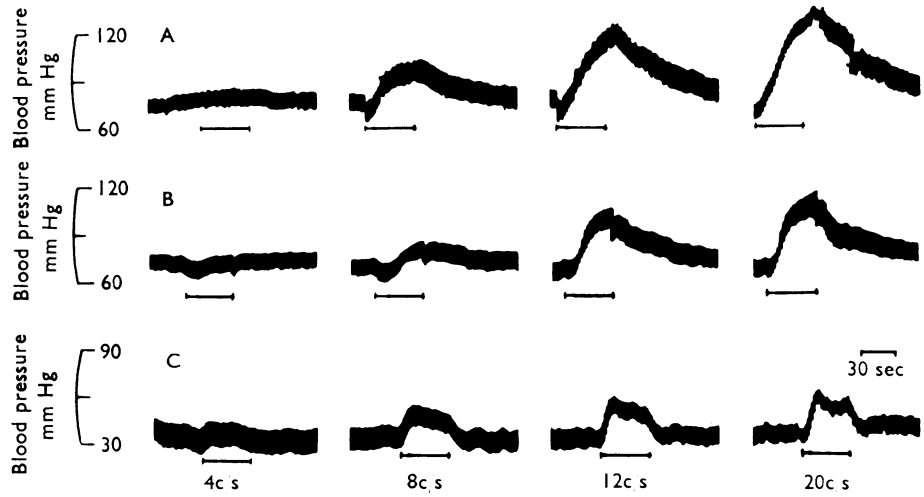

FIG. 2. Effects of guanethidine pretreatment on the blood pressure response to different rates (4, 8, 12 and 20 c/s) of splanchnic nerve stimulation in intact rats (A, guanethidine 20 mg/kg), in rats demedullated by expression (B, guanethidine 20 mg/kg) and by grafting (C, guanethidine 7 mg/kg).

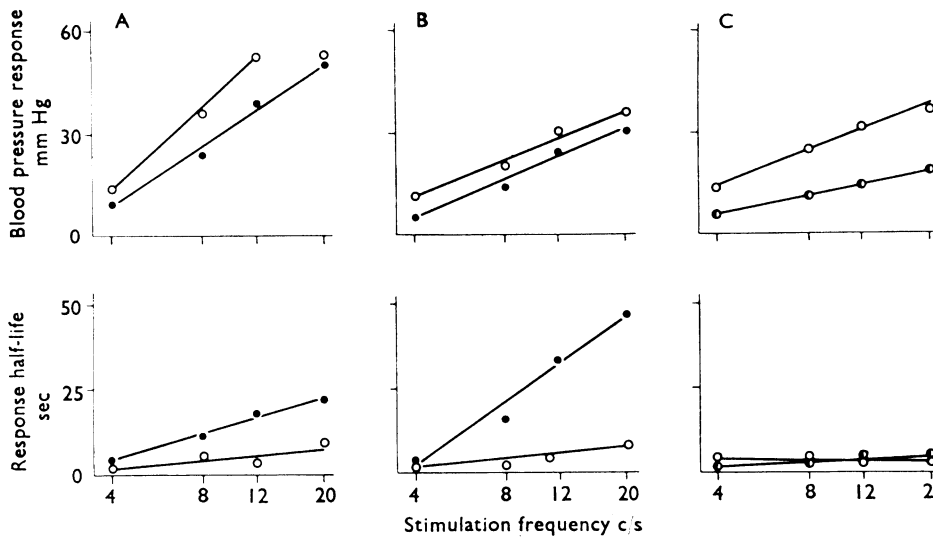

FIG. 3. Effects of guanethidine pretreatment on the frequency-response curve (upper tracing) and on the frequency-half-life curves (lower tracing) in intact rats (A), in rats adrenal demedullated by expression (B) and by grafting (C). ○, Untreated rats ; ●, guanethidine 20 mg/kg; ◖, guanethidine 7 mg/kg.

($F=5.5$; $P<0.025$). Moreover the blood pressure increase and return to pre-stimulation level were slower in treated than in control rats—that is, the duration of the pressor response was increased. This was most marked with the highest rates of stimulation (Fig. 2A). The slope of the regression of the half-life of the response on the log of frequency of stimulation was significantly higher ($F=12.7$; $P<0.001$) in the rats pretreated with guanethidine than in normal untreated rats.

In rats demedullated by expression and pretreated with guanethidine, arterial blood pressure was 43.3 ± 4.6 mm Hg ; this figure was not significantly different from that observed in the corresponding untreated rats ($P>0.05$). The effects of guanethidine pretreatment in rats demedullated by expression were similar to those observed in intact rats—that is, a slight reduction of the blood pressure response and an increase in its duration (Fig. 2B, Fig. 3 and Table 1). This is substantiated by the significant shift to the right ($F=4.9$; $P<0.05$) of the frequency-response curve without changes in the slope ($F=0.07$; $P>0.05$) and by the significantly higher regression coefficient of the half-life of the response on the log of the stimulation frequency observed, after guanethidine administration, in rats demedullated by expression ($F=63.7$; $P<0.001$) (Table 1).

Rats demedullated by adrenalectomy with cortical grafting and pretreated with guanethidine 20 mg/kg were unable to withstand the surgical procedures used in the present experiments: blood pressure before stimulation in these animals was always less than 30 mm Hg and splanchnic nerve stimulation did not raise arterial pressure. With a smaller dose of guanethidine (7 mg/kg subcutaneously, 16–20 hr before the test) the average prestimulation blood pressure was 43.0 ± 2.6 mm Hg ; this figure was not significantly different from that observed in the corresponding untreated controls and in guanethidine pretreated rats demedullated by expression ($P>0.05$). Rats with grafts pretreated with guanethidine 7 mg/kg had a less marked rise in arterial pressure on splanchnic stimulation and the time course of this response was similar to that of untreated rats demedullated by the same technique (Fig. 2C). Statistical analysis of these results indicates that guanethidine reduced the slope of the frequency-response curve ($F=7.2$; $P<0.025$). No significant regression of the half-life of the response on the log of the frequency of stimulation was present in rats demedullated by adrenalectomy with cortical grafting, pretreated with guanethidine (Fig. 3 and Table 1). Comparison of results in demedullated rats shows that, even with the lower dose, guanethidine caused a significantly greater reduction of the pressor response to splanchnic stimulation in animals with grafts (difference in the slope: $F=12.0$; $P<0.001$).

Discussion

In the present experiments the prestimulation levels of arterial pressure in all experimental groups were considerably lower than that normally observed in the rat anaesthetized with urethane (80–120 mm Hg). This is undoubtedly due to the surgical trauma involved in the preparation of the splanchnic nerve. The absence of statistically significant differences between prestimulation blood pressure levels in the various experimental groups indicates that the different reactivity to splanchnic nerve stimulation, observed in the present experiments, cannot be considered to be related to differing degrees of surgical trauma.

The linear regression of the hypertensive response on the log of the stimulation frequency has been described by Brody (1966) in his perfusion experiments ; in

the present observations, the covariance analysis of the calculated regression shows that the pressor response to splanchnic stimulation is significantly reduced in adrenal demedullated rats. Various explanations may be proposed. The possibility exists that damage to the splanchnic nerve occurred during the intervention on the adrenals ; this, however, seems to be excluded by previous observations that adrenal demedullation is followed by an increase in the urinary output of noradrenaline ; moreover, the same rise in the urinary excretion of this mediator is observed in intact as well as in adrenal-demedullated rats after an acute pharmacological stress (Biscardi, Carpi & Orsingher, 1964). Adrenocortical insufficiency reduces the reactivity of the cardiovascular system to sympathetic stimulation (Ramey & Goldstein, 1957) ; a degree of cortical insufficiency in demedullated rats may explain the reduced pressor response to splanchnic stimulation in these animals. No direct evidence of this interpretation is given by the present results. On the contrary, adrenal demedullation was well tolerated by the animals and no maintenance therapy was necessary. Furthermore, the prestimulation blood pressure levels were the same in demedullated and in control animals. Blood pressure levels below 30 mm Hg and an absence of pressor reactions to splanchnic stimulation were observed only in adrenalectomized rats maintained with saline or with incomplete replacement therapy (deoxycorticosterone, 1 mg/kg per day or cortisone 2 mg/kg per day), whereas in adrenalectomized rats maintained with both steroids, the frequency-response curve of splanchnic stimulation was similar to that observed in adrenal demedullated rats (Cartoni & Carpi, 1968). Thus it seems reasonable to conclude that, under the present experimental conditions, the adrenal medulla contributes significantly to the pressor response ; this contribution is particularly consistent at the highest rates of stimulation as shown by the significant reduction in the slope of the frequency response curve of adrenal demedullated rats.

This conclusion must be considered in regard to the data concerning the half-life of the response. The regression of the half-life on the log of stimulation frequency observed in intact untreated rats may be simply explained by the obvious fact that a correlation exists between the duration and the magnitude of the pressor response. It has been shown, however, that arterial hypertension induced by splanchnic nerve stimulation consists of a fast component due to neurogenic vasoconstriction and a slower and more delayed humoral component due to release of catechol amines from the suprarenal chromaffin tissue (De Vleeschhouwer, 1935 ; Nickerson & Goodman, 1947). It is now evident that, when the duration of a composite pressor response of this type is evaluated on the basis of its half-life, an increase of the relative contribution of the slow component to the overall pressor response will prolong the half-life of this response. As a consequence, the increasing contribution of the humoral component to the pressor response, brought about by the increase in the rate of stimulation, may explain the regression of the half-life on the frequency of stimulation observed in intact rats, and the absence of a significant regression in rats which were demedullated by means of cortical transplantation.

On the basis of the foregoing discussion, an intermediate type of response has been obtained in rats demedullated by expression: whereas the frequency-response curve indicates that there is no significant humoral contribution to the pressor response, the presence of a slow component in the response is supported by the half-life-frequency curve obtained in these animals. A more detailed explanation

of this equivocal pattern of response could be demonstrated if a reliable analysis of the time course interrelations between the fast and the slow component could be made by means of a bi-exponential analysis of the decay of the pressor curve. Other data, however, indicate that, in rats demedullated by expression, there is an intermediate type of adrenomedullary activity as compared with that of intact rats or of rats demedullated with cortical grafting. Thus adrenal demedullation by expression is followed by a fall in the urinary excretion of adrenaline which is similar to that observed after adrenal demedullation with cortical grafting (Del Basso, Rusca & Carpi, 1967). During a phase of sympathetic hyperactivity, an increase in adrenaline excretion is observed in rats demedullated by expression (Biscardi, Carpi & Orsingher, 1964) whereas it is absent in rats demedullated with cortical grafting (Del Basso, Rusca & Carpi, 1967).

These conclusions bear a definite relation to the discussion of the effects exerted by guanethidine. It has been previously reported that this drug, in spite of its inactivating action on sympathetic nerve endings (Costa, Boullin, Hammer, Vogel & Brodie, 1966), fails to inhibit the arterial hypertension induced by splanchnic nerve stimulation in the cat (Abercrombie & Davies, 1963). Two aspects of the pharmacological action of guanethidine explain this fact ; first, guanethidine does not inhibit the release of mediators from the adrenal medulla (Cass & Spriggs, 1961 ; Athos, McHugh, Fineberg & Hilton, 1962), and second, the drug potentiates the peripheral action of adrenaline and noradrenaline (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960). As a consequence, the normal neurogenic component of the pressor response to splanchnic stimulation is replaced by the humoral component in the drug-treated animal (Abercrombie & Davies, 1963). The results obtained in intact rats fit with this interpretation. In fact, guanethidine, although reducing the absolute values of the rise in blood pressure induced by splanchnic stimulation, clearly modifies the time course of this response ; the more extended half-life values of the pressor response, and the steeper regression of the half-life on the stimulation frequency observed in rats pretreated with guanethidine may be considered to reflect the absolute or the relative prevalence of the humoral component of the pressor response. If this conclusion is correct, the more intensive inhibition of the pressor response without changes in the half-life, induced by guanethidine in rats with grafted adrenal cortex, could be considered a further evidence of the absence of adrenomedullary activity in these animals. Conversely, the less intensive inhibition and the increase in the half-life of the pressor response, observed in adrenal demedullated rats by expression pretreated with guanethidine, should indicate that a certain level of this activity is still present in these rats. The differences in the toxicity of guanethidine in the two types of adrenal demedullated rats may be explained on the basis of this difference in adrenomedullary activity.

From the foregoing discussion it can be concluded that the persistence of a certain degree of adrenomedullary function in rats demedullated by expression suggests that the significance of the results obtained with this method of demedullation must be considered with caution. Finally the significant contribution of the adrenal medulla to the blood pressure response to splanchnic nerve stimulation in intact rats may not conflict with the observation (Brody, 1966) that the effects of splanchnic nerve stimulation on perfusion pressure in hind quarters of the rat are not significantly different in intact rats and in rats adrenal demedullated by expression. In fact, perfusion pressure is not influenced by changes in cardiac perform-

ance induced by the sympathetic mediators released from the adrenal medulla. On the other hand, the cardiac effects of adrenomedullary secretion certainly play a part in the blood pressure responses studied in the present experiments. From a more general point of view, this can also explain why the contribution of the adrenal medulla to the effects exerted by the sympathetic system (Cannon, 1928) has been questioned on the basis of studies principally concerned with the reaction of the peripheral vascular system (Celander, 1954).

REFERENCES

ABERCROMBIE, G. F. & DAVIES, B. N. (1963). The action of guanethidine with particular reference to the sympathetic nervous system. *Br. J. Pharmac. Chemother.*, **20**, 171–177.

ATHOS, W. J., MCHUGH, B. P., FINEBERG, S. E. & HILTON, J. C. (1962). The effects of guanethidine on the adrenal medulla. *J. Pharmac. exp. Ther.*, **137**, 229–234.

BISCARDI, A. M., CARPI, A. & ORSINGHER, O. A. (1964). Urinary excretion of catechol amines in the rat after their liberation by reserpine or dexamphetamine. *Br. J. Pharmac. Chemother.*, **23**, 529–539.

BRODY, M. J. (1966). Effect of adrenal demedullation on vascular responses after immunological sympathectomy. *Am. J. Physiol.*, **211**, 198–202.

CANNON, W. B. (1928). Die Notfallsfunktionen des sympathico-adrenalen System. *Ergebn. Physiol.*, **27**, 380–406.

CARTONI, C. & CARPI, A. (1968). Adrenocortical and adrenomedullary factors influencing the blood pressure response to splanchnic stimulation in the rat. *Ann. Ist. Super. Sanità*, in the Press.

CASS, R. & SPRIGGS, T. L. B. (1961). Tissue amine levels and sympathetic blockade after guanethidine and bretylium. *Br. J. Pharmac. Chemother.*, **17**, 442–450.

CHANG, C. C., COSTA, E. & BRODIE, B. B. (1965). Interaction of guanethidine with adrenergic neurons. *J. Pharmac. exp. Ther.*, **147**, 303–312.

CELANDER, O. (1954). The range of control exercised by the " sympathico-adrenal system." *Acta physiol. scand.*, **32**, suppl. 116, 1–132.

COSTA, E., BOULLIN, D. J., HAMMER, W., VOGEL, W. & BRODIE, B. B. (1966). Interactions of drugs with adrenergic neurons. *Pharmac. Rev.*, **18**, 577–597.

DEL BASSO, P., RUSCA, G. & CARPI, A. (1967). Origine extramidollare dell'adrenalina. *Boll. Soc. ital. Biol. Sper.*, *xliii*, fasc. 20 bis., 103.

DE VLEESCHHOUWER, G. (1935). Au sujet de l'action du diéthylaminométhyl-3-benzodioxane (F. 883) et du pipéridométhyl-3-benzodioxane (F. 933) sur le système circulatoire. *Archs int. Pharmacodyn. Thér.*, **50**, 251–295.

HILLARP, N. A. & HÖKFELT, B. (1955). Histochemical demonstration of noradrenaline and adrenaline in the adrenal medulla. *J. Histochem. Cytochem.*, **3**, 1–5.

MAXWELL, R. A., PLUMMER, A. J., SCHNEIDER, F., POVALSKI, H. & DANIEL, A. I. (1960). Pharmacology of [2-(octahydro-1-azocinyl)-ethyl]-guanidine sulfate (Su-5864). *J. Pharmac. exp. Ther.*, **128**, 22–29.

NICKERSON, M. & GOODMAN, L. S. (1947). Pharmacological properties of a new adrenergic blocking agent: N,N-dibenzyl-β-chloroethylamine(Dibenamine). *J. Pharmac. exp. Ther.*, **89**, 167–185.

RAMEY, E. R. & GOLDSTEIN, M. S. (1957). The adrenal cortex and the sympathetic nervous system. *Physiol. Rev.*, **37**, 155–195.

SKELTON, F. R. (1959). Adrenal regeneration and adrenal regeneration hypertension. *Physiol. Rev.*, **39**, 162–182.

SNEDECOR, G. W. (1956). *Statistical Methods*, 5th ed. Ames, Iowa: Iowa State University Press.

(*Received April* 26, 1968)