hibit the renal effect of parathyroid hormone.

*Summary.* Actinomycin D inhibits the hypercalcemia induced by large doses of parathyroid extract or Vit. D. It also alters the bone changes induced by these agents. The data thus indicate that these agents may act by inducing synthesis of new enzymes in bone induced by DNA directed RNA which in turn enzymatically degrade bone.

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## Inhibition of Autoregulation of Renal Blood Flow by SKF 525-A and Fluoroacetate.* (29502)

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The ability of the kidney to maintain renal blood flow and glomerular filtration at relatively constant rates over a wide range of systemic arterial pressures has been termed autoregulation(1,2). To explain this phenomenon various mechanisms, both passive and active, have been proposed. Of the passive mechanisms, a cell separation theory has been advanced by Pappenheimer(3) and an intrarenal pressure theory by Winton(4) and Hinshaw(5). An active mechanism is suggested by the myogenic theory of Miles *et al* (6) and Waugh and Shanks(7) which implies that autoregulation occurs as a result of constriction of renal vascular smooth muscle in response to the stimulus of an increase in distending pressure. Support for this active mechanism has in part been obtained using chemical inhibitors(6,7). The inhibitory action of β-diethylaminoethyl-diphenylpropylacetate hydrochloride (SKF 525-A) and sodium fluoroacetate described here provides further evidence for an active myogenic mechanism of autoregulation.

*Methods.* Twenty-five male or female mongrel dogs, 18 to 25 kg, were used. Pentobarbital sodium, 30 mg/kg, was administered intravenously to anesthetize the animals and supplemental doses were given as necessary. The trachea was cannulated to insure an adequate airway. Systemic blood pressure was monitored from a carotid artery using a Statham arterial pressure transducer. The left kidney was exposed retroperitoneally by

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a flank incision. The left renal artery was carefully dissected free from its surrounding tissue and nerve plexus to enable cannulation of the vessel without interruption of the nerve supply. The artery was cannulated and perfused with blood from a previously cannulated femoral artery using a Sigmamotor pump (model T6S). Renal blood supply was interrupted for no more than 2 minutes and in most experiments less than one minute. Perfusion pressure was recorded with a second transducer attached to a sidearm of the renal artery cannula just proximal to the kidney. Flow was regulated initially so that perfusion pressure was about 20 mm Hg less than systemic blood pressure. Heparin sodium (5 mg/kg) was used as an anticoagulant and was administered intravenously just prior to cannulation of the renal artery but after all other surgical procedures. Systemic blood pressure and perfusion pressure were recorded continuously on a Gilson direct writing oscillograph.

Saline was infused (1 ml/min) continuously into the renal artery *via* the cannula using a Harvard infusion-withdrawal pump (model 600-900). Drug infusions (SKF 525-A or sodium fluoroacetate in saline) replaced the saline infusion at appropriate times. With this type of administration no systemic effects of the agents employed were apparent. Norepinephrine (0.5 $\mu$g) was injected into the renal cannula. Pressure-flow curves were obtained before, during and after infusion of the drugs. Flow was increased by serially increasing the stroke rate of the pump in unit increments. Changes in renal vascular resistance were calculated by dividing perfusion pressure (mm Hg) by the renal blood flow (ml/min). The values obtained are expressed as resistance units.

Animals which received reserpine (0.2 mg/kg) were given this agent intramuscularly 48 and 24 hours prior to experiment.

Data were analyzed statistically by either Duncan's new multiple range test using a completely randomized block design or by the student "t" test(8). Variability is expressed as standard error of the mean.

*Results.* In these experiments renal autoregulation was produced by increasing the blood flow rate through the kidney. Autoregulation was considered to be present when a unit increase in flow resulted in a greater than unit increase in perfusion pressure. An example of a pressure-flow curve illustrating autoregulation is seen in the first portion of Fig. 1. The remainder of Fig. 1 illustrates that infusion of SKF 525-A resulted in an immediate loss of the autoregulatory response, and that the response in large part returned after the drug infusion was terminated. Pressure-flow curves from the 13 experiments in which SKF 525-A was used to block autoregulation are shown in Fig. 2. A summary of the renal vascular resistance changes obtained with SKF 525-A in these 13 experiments is shown in Table I. Autoregulation (as defined by a net increase in renal vascular resistance) was observed during the control period of each experiment. During infusion of SKF 525-A renal vascular resistance decreased with increasing renal blood flow. Autoregulation showed evidence of return 2 to 11 minutes following cessation of drug infusion. Peak return (Table I) was found 2 to 18 minutes after infusion. Responsiveness of the renal vasculature to norepinephrine was significantly depressed during administration of SKF 525-A and returned after the infusion was terminated. In 4 other experiments renal autoregulation was not observed, *i.e.*, renal vascular resistance decreased by an average of 0.31 $\pm$ 0.12 resistance units as flow was increased. Pressure-flow curves taken during SKF 525-A infusion

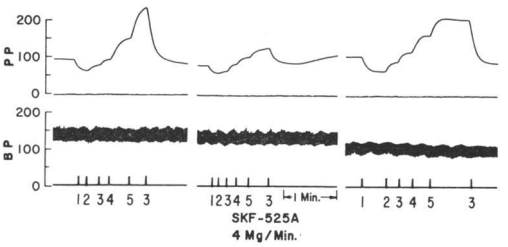


FIG. 1. Effect of SKF 525-A on autoregulation in the perfused kidney. Perfusion pressure (PP) and systemic blood pressure (BP) are calibrated in mm Hg. At the numbers below the tracing, flow to the kidney was changed. Flow rates (ml/min/g kidney weight) represented by these numbers are: 1, 1.5; 2, 2.2; 3, 2.9; 4, 3.5; 5, 4.2. Left trace, before drug; middle trace, during SKF 525-A; right trace, 18 min after drug.

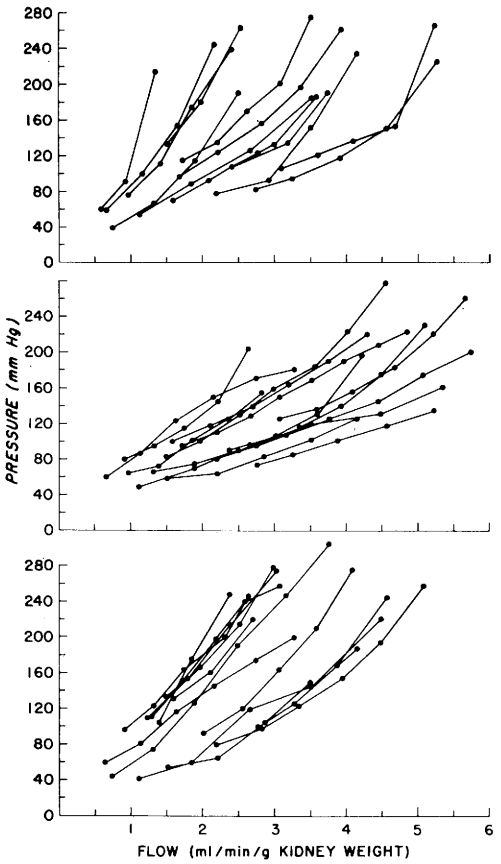


FIG. 2. Effect of SKF 525-A on pressure flow curves in the kidney. Each line represents a curve from a single experiment. Upper panel curves are from control periods. Middle panel curves are from periods of infusion of SKF 525-A. Lower panel curves were taken at time of peak return.

in these experiments showed that the drug produced a further decrease in vascular resistance (0.47 ± 0.15) as a result of increased blood flow. As in the experiments showing autoregulation, constrictor responses produced by norepinephrine were significantly reduced.

Renal autoregulation was also inhibited by the infusion of sodium fluoroacetate at a dose of 2 mg/min for 15 min. In contrast to the transient effect seen with SKF 525-A, the loss of both autoregulation and the responsiveness to norepinephrine persisted throughout the experiment, and the effect of fluoroacetate on these parameters appeared to become progressively greater with time (Table I).

SKF 525-A and fluoroacetate had little effect on the existing perfusion pressure. The slight falls in pressure produced by each agent (10 ± 6 mm Hg for SKF 525-A and 8 ± 3 mm Hg for fluoroacetate) were not statistically significant.

Four animals were pretreated with reserpine for 2 days prior to the experiment. As seen in Table I all of these animals exhibited the autoregulatory response as renal blood flow was increased.

*Discussion.* In this study 2 different types of metabolic inhibitors, SKF 525-A and fluoroacetate, were found to abolish the increase in renal vascular resistance which is associated with increased renal blood flow. SKF 525-A has been reported to inhibit hepatic enzyme systems responsible for biotransformation of various drugs(9). It has also been reported to inhibit sodium transport by the kidney(10), an effect which was quite transient, observable only during infusion of the drug. Fluoroacetate is a long acting metabolic inhibitor which blocks the tricarboxylic acid cycle(11).

The fact that a metabolic inhibitor is able to block autoregulation suggests that the mechanism of the autoregulatory response involves an active rather than a passive process. If renal autoregulation were the result of mechanical processes such as cell separation(3) or increased extravascular pressure (4,5), these processes would not be expected to be affected by addition of a metabolic inhibitor. Since these inhibitors were effective antagonists of autoregulation, it may be concluded that the action of these agents probably involves blockade of an active myogenic response. This suggestion is supported by the data obtained with norepinephrine. Both SKF 525-A and fluoroacetate significantly depressed the ability of renal vascular smooth muscle to respond to norepinephrine. In the case of both inhibitors the depression of responsiveness to norepinephrine paralleled the blockade of autoregulation.

Since the metabolic inhibitors were depressing smooth muscle, one might have expected these agents to decrease the existing resistance to blood flow in the kidney. Although a small decrease in perfusion pressure

TABLE I. Effect of SKF 525-A, Fluoroacetate and Reserpine on Renal Vascular Resistance During Changes in Blood Flow and on Renal Vascular Responsiveness to Norepinephrine.

| Procedure | | N | Control period (saline infusion) | During drug infusion | Following drug (saline infusion) |
|---|---|---|---|---|---|
| SKF 525-A, 4 mg/min, infusion into renal artery | Change in resistance† (resistance units) | 13 | .38 ± .07* | −.17 ± .08 | .15 ± .10* (2-18 min)‡ |
| | Norepinephrine (.5 μg): change in perfusion pressure (mm Hg) | 9 | 113 ± 11* | 27 ± 9 | 94 ± 9* (2-18 min)‡ |
| Sodium fluoroacetate, 2 mg/min, infusion into renal artery | Change in resistance (resistance units) | 4 | .47 ± .17 | −.17 ± .17* | −.24 ± .08* (20 min after) |
| | Norepinephrine (.5 μg): change in perfusion pressure (mm Hg) | 4 | 163 ± 24 | 65 ± 17* | 27 ± 5* (20 min after) |
| Reserpine, .2 mg/kg, 48 and 24 hr prior to experiment | Change in resistance (resistance units) | 4 | .56 ± .12 | — | — |
| | Norepinephrine (.5 μg): change in perfusion pressure (mm Hg) | 4 | 94 ± 21 | — | — |

* In any one row, any 2 values marked with an asterisk do not differ significantly. A value with an asterisk and a value without an asterisk are significantly different ($p<.05$). Statistical analysis was performed using Duncan's new multiple range test following analysis of variance using a completely randomized block design.

† Change in resistance represents the difference between the resistances at the first and last flow settings of the pressure-flow curve.

‡ Peak return. N = No. of animals.

was produced by each agent this decrease was not significant. This is to be expected, since renal vascular smooth muscle possesses little neurogenic or myogenic tone and thus has relatively little dilator capacity(12). SKF 525-A can be shown to reduce vascular resistance in the hind quarters of the dog (unpublished observation) a vascular bed which possesses a considerable component of constrictor tone. Although it is apparent that the contractile properties of vascular smooth muscle are depressed by the inhibitors used in this study, no definitive statement can be made concerning the mechanism by which this depressant effect is brought about. It is not known whether depression results from interference with essential metabolic pathways or from a "non-specific" depression of smooth muscle.

Adrenergic blocking agents have been previously shown to be essentially without effect upon autoregulation of renal blood flow(7, 13). On this basis it would appear that norepinephrine does not play an important role in mediating the increase in renal vascular resistance which accompanies increased renal blood flow. Since reserpine is known to deplete tissue stores of vasoactive materials other than norepinephrine, *e.g.* serotonin(14) it seemed worthwhile to determine if autoregulation might be affected by pretreatment with this agent. Since autoregulation was present in the kidneys of animals treated with reserpine, these experiments failed to shed light on the nature of a possible chemical mediator of renal autoregulation.

It should be emphasized that the inhibitory action of SKF 525-A was rapidly reversible. This property of the agent would appear to make it a useful tool for further studies on the mechanisms which contribute to renal autoregulation.

*Summary.* Autoregulation of renal blood flow and responsiveness to norepinephrine were found to be inhibited when SKF 525-A or sodium fluoroacetate were administered *via* the renal artery. SKF 525-A was effective only during the period of infusion. The brevity of action of this compound would appear to make it a useful tool in studies on autoregulation of renal blood flow. In contrast to SKF 525-A, sodium fluoroacetate produced an irreversible blockade. Pretreatment with reserpine did not affect the autoregulatory response. These data are interpreted as further evidence for an active myo-

genic mechanism in autoregulation of renal blood flow.

The authors are indebted to Smith Kline and French Laboratories for the SKF 525-A used in these experiments.

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## Plasma Free Fatty Acids after 2-Deoxy-D-Glucose in Intact, Adrenalectomized, Spinal and Hypophysectomized Rat.* (29503)

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Administration of 2-Deoxy-D-Glucose (2-DG) is followed by marked hyperglycemia in rat(1), dog(2) and man(3) and also a striking increase in secretion of adrenaline and noradrenaline from the adrenal medulla (4). The primary mechanism underlying these effects seems to be inhibition of intracellular glucose utilization(1). It is probable that 2-DG induced cellular glucopenia within the central nervous system, which triggers the release of catecholamines, is largely responsible for the hyperglycemia(4). Since sympathomimetic amines as well as glucose influence the release of free fatty acids (FFA) both *in vivo* and *in vitro* (see 5 for review), we investigated the effect of 2-DG on plasma FFA and its relation to the release of adrenal medullary hormones. In addition, studies were performed to explore the role of the adrenal cortex and the pituitary in this connection.

*Materials and methods.* Inbred, albino rats of both sexes, 200-300 g, maintained on a standard diet, were used. All experiments were started AM, at which time food was removed but water allowed *ad libitum.* Adrenalectomy was performed *via* 2 lumbar incisions 3 days before administration of 2-DG and the rats were maintained on 0.9% saline.. Parapharyngeal hypophysectomy was performed 6 days prior to 2-DG and adrenal denervation by spinal cord transsection at level C-7, about one hour before an experiment. Ether anesthesia was used for all operations. 2-DG (Aldrich Chemical Co., Milwaukee, Wisc.) was obtained through courtesy of Cancer Chemotherapy National Service Center, NIH, Bethesda. In all instances 2-DG (100 mg/100 g b.w.) was administered subcutaneously (s.c.) in water solution (100

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