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# THE FORMATION OF AN ISOPRENALINE-LIKE SUBSTANCE FROM ADRENALINE

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Intravenous injections of adrenaline, but not those of noradrenaline, caused the appearance of a substance which resembled isoprenaline in $R_F$ value and pharmacological activity in blood withdrawn from the lower abdominal aortae of chloralosed cats rested after acute adrenalectomy and induction of lasting block in autonomic ganglia with hexamethonium. The formation of the isoprenaline-like compound was prevented by pretreatment with pyrogallol but not by pretreatment with reserpine, cocaine, phentolamine, dibenamine, harmaline, or iproniazid. The liver proved a main site of the origin of this isoprenaline-like substance.

Trace quantities of a substance which resembled isoprenaline in its pharmacological actions and $R_F$ values were found in saline extracts of adrenal glands from cats, monkeys and man (Lockett, 1954); they appeared in blood perfusing cat heart–lung preparations when the upper thoracic sympathetic chains were stimulated (Lockett, 1957). Traces of a similar substance were detected in the plasma of blood withdrawn from the lower abdominal aortae of cats during pressor responses to intravenous injections of adrenaline; neither pretreatment of the animals with cocaine, 7 mg/kg subcutaneously, nor the intravenous injection of phentolamine, 2 mg/kg, prevented the appearance of the trace compound (Lockett, 1959).

The present work has confirmed and extended the hypothesis (Lockett, 1959) that the isoprenaline-like compound arises as a metabolite of adrenaline, and has shown that the liver is the major site of the formation of the metabolite. Conditions under which maximum yields of isoprenaline-like substance are obtained have been studied in expectation that the knowledge gained will lead to the isolation of this trace compound in quantity sufficient for its chemical identification.

## METHODS

Anaesthesia was induced with ether and maintained by intravenous injection of chloralose 1.0%, 7 ml./kg, in male, female, or neuter cats. Tracheal and femoral venous cannulae were inserted, the adrenals were excluded from the circulation by purse-string ligatures and hexamethonium bromide was injected, 10 mg/kg both intravenously and subcutaneously, 30 to 40 min before every experiment. Heparin (Liquemin, Roche, 100,000 units/kg) was injected intravenously and the blood pressure was recorded from a carotid arterial cannula on all occasions. The quantities of hexamethonium used were regularly shown to block transmission of electrical excitation of an ascending cervical sympathetic chain to the corresponding nictitating membrane for the duration of the subsequent experiments.

In experiments designed for the study of the sympathetic amines present in lower aortic blood all injections were made into the right femoral venous cannula and blood samples were withdrawn through a polythene tube inserted into the aorta through the right femoral artery so that the tip lay 0.5 to 2.0 cm above the bifurcation. Ten minutes after the collection of a resting sample of arterial blood an intravenous injection of adrenaline or noradrenaline was made, and an arterial blood sample was collected throughout the response of the mean arterial pressure. The whole process was repeated 20 min after the effects of this injection had disappeared. The procedure differed when cocaine (7 mg/kg subcutaneously), phentolamine (2 mg/kg intravenously), dibenamine (20 mg/ml. propylene glycol, 1.0 ml./kg), harmaline (2.5 mg/kg intravenously), pyrogallol (25 mg/kg intravenously), or iproniazid (5 ml. 0.1 M in 0.9% NaCl/kg intraperitoneally) was used. The effect of the drug on the response of the mean arterial pressure to adrenaline was demonstrated before the first blood sample was collected. Animals pretreated with reserpine received 5 mg/kg of the hydrochloride (intramuscularly) 36 hr before anaesthesia was induced.

In experiments designed to demonstrate the major systemic site or sites of the liberation or formation of the isoprenaline-like substance, the procedure differed only in the following points. First, injections of adrenaline were made through polythene tubes inserted either into the hepatic artery (by retrograde cannulation of the splenic) or into the main splenic artery (by retrograde cannulation of a branch more centrally situated than the cannulated artery) or into a common iliac artery (by retrograde cannulation of the opposite femoral artery) or into the portal vein (by retrograde cannulation of the splenic vein and ligature of the splenic artery). Secondly, samples of venous blood were withdrawn in one experiment from the inferior vena cava by means of a polythene tube inserted through the right external jugular vein, the right atrium and thoracic inferior cava until the tip lay just above the entry of the hepatic veins. Alternatively, blood was withdrawn from a common iliac vein by retrograde cannulation of the opposite femoral vein. Electrical stimulation of the hepatic nerves was effected by means of shielded platinum electrodes delivering rectangular pulses of 0.5 msec duration at a rate of 20 per sec from a 2-volt source.

Blood samples of 5 to 8 ml. were collected into cooled heparinized tubes and the plasmas were separated without delay. Separate plasma pools were made from blood samples collected at rest and during responses to either adrenaline or noradrenaline.

The methods used to prepare protein-free extracts of plasma, for phenol-hydrochloric acid chromatography, and elution have been previously described (Lockett, 1957). The amines were separated chromatographically and assayed biologically in the eluates. Adrenaline was determined by inhibition of the response of the rat uterus to a fixed dose of acetylcholine, submaximal in effect (Gaddum, Peart & Vogt, 1949), and isoprenaline-like activity was assayed both by its depressor action on the arterial pressures of rats anaesthetized with pentobarbitone (Nembutal, Abbott Laboratories), 0.1 ml./100 g intraperitoneally, and on the rat uterus as described above.

*Drugs.* Heparin and iproniazid phosphate (Roche Products), reserpine and phentolamine (Ciba Laboratories), cocaine hydrochloride and adrenaline acid tartrate (Burroughs Wellcome), noradrenaline acid tartrate and harmaline (L. Light & Co.), isoprenaline hydrochloride (Bayer Products), hexamethonium bromide (May & Baker), pyrogallol (Hopkin & Williams), dibenamine (Smith, Kline & French Laboratories), and pentobarbitone sodium (Veterinary Nembutal, Abbott Laboratories) were obtained commercially.

## RESULTS

No isoprenaline-like activity was demonstrable in plasma separated from blood withdrawn from the lower abdominal aortae of cats anaesthetized with chloralose which had been rested for 30 to 40 min after exclusion of the adrenal glands from the circulation and the induction of lasting block to transmission in autonomic ganglia. The resting plasma did, however, contain measurable concentrations of

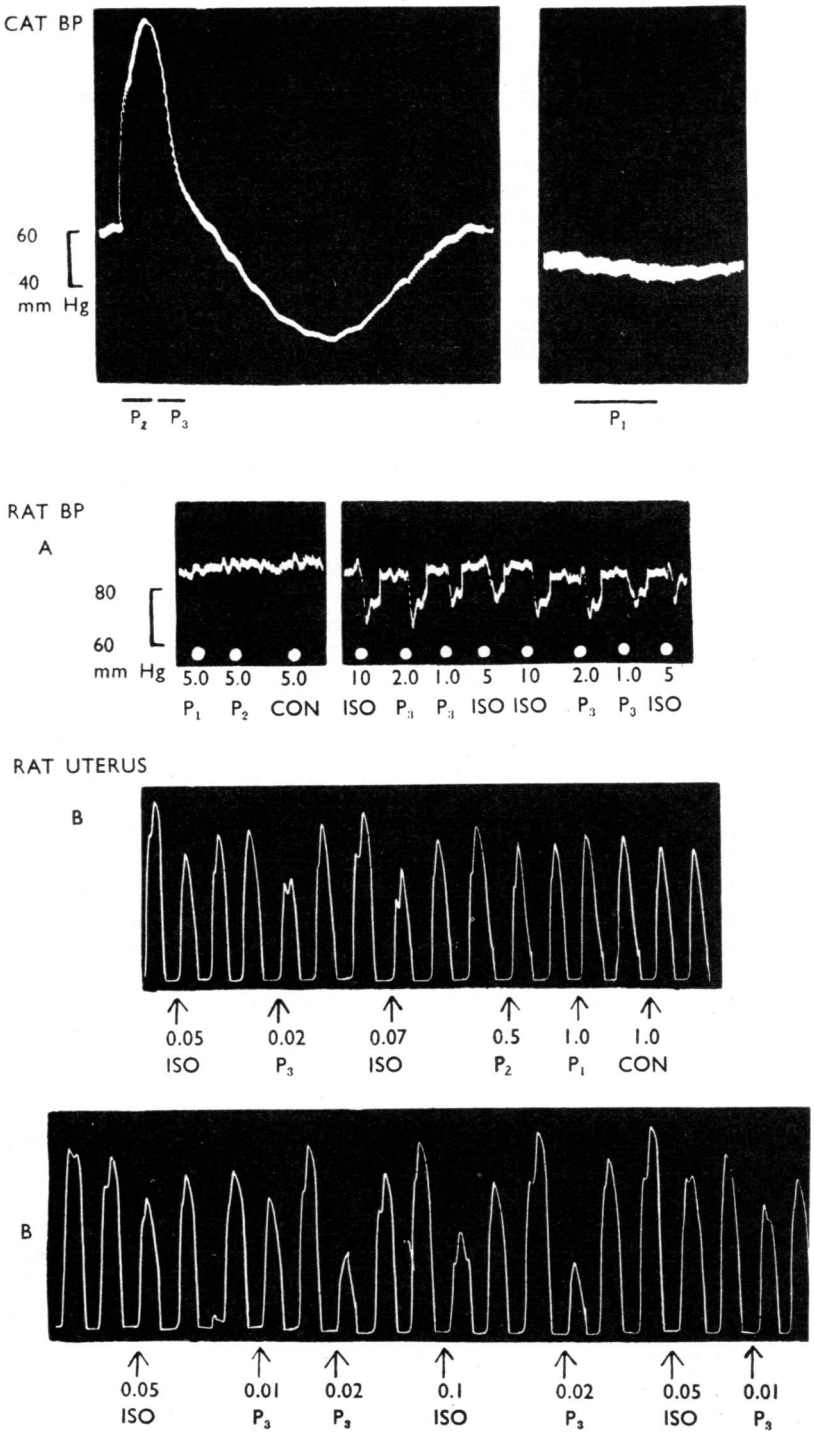


Fig. 1. Isoprenaline-like activity is demonstrable in the plasma of aortic blood taken from acutely adrenalectomized cats treated with hexamethonium during pressor responses to intravenous adrenaline. Above, record of the arterial pressure of cat during the collection of 10 ml. blood samples at rest ($P_1$) and during a response to 6 $\mu$g/kg adrenaline given intravenously ($P_2$ and $P_3$). Below, assays of isoprenaline-like activity separated chromatographically from the samples of cat plasma; A, by depression of the mean arterial pressure of a rat under pentobarbitone anaesthesia; B, by inhibition of submaximal effects of 0.2 $\mu$g acetylcholine on a quiescent rat uterus in 5 ml. aerated de Jalon fluid at 28° C. Doses: isoprenaline, ng (ISO); plasma eluates in ml. equivalent of original plasma ($P_1$, $P_2$, $P_3$); control eluate of $R_F$ value intermediate between adrenaline and isoprenaline from plasma $P_3$ (CON).

adrenaline and noradrenaline (Lockett & Eakins, 1960). Isoprenaline-like activity (Fig. 1) appeared in the plasma during pressor responses to adrenaline but not during those to noradrenaline (Table 1).

TABLE 1

CONCENTRATIONS OF ISOPRENALINE-LIKE COMPOUND APPEARING IN PLASMA DURING PRESSOR RESPONSES TO INTRAVENOUS INJECTIONS OF ADRENALINE AND NORADRENALINE

The samples of blood were withdrawn from the lower aortae of cats anaesthetized with chloralose and rested for 30 to 40 min, after exclusion of the adrenal glands from the circulation and induction of lasting block in ganglia with hexamethonium

| | | Plasma concentration, μg/100 ml. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Adrenaline | | Noradrenaline | | Isoprenaline | |
| Treatment | No. of cats | Resting | Responding | Resting | Responding | Resting | Responding |
| Adrenaline (4–8 μg/kg) | (5) | 0·07±0·01 | 1·1±0·25 | — | — | <0·005 | 0·64±0·20 |
| Noradrenaline (2·7–7 μg/kg) | (6) | — | — | 1·68±0·69 | 10·7±2·05 | <0·010 | <0·005 |

*Effect of inhibitors of the amine oxidase and O-methyl transferase on the concentrations of amines in aortic plasma.* Pretreatment of cats with cocaine, pyrogallol, harmaline and iproniazid, in concentrations which increased the pressor effect of a fixed intravenous dose of adrenaline, increased the concentrations of adrenaline in the plasma of blood withdrawn from the lower abdominal aortae of the resting animals. Whereas isoprenaline-like activity became readily detectable at rest in the plasma of animals pretreated with cocaine, harmaline and iproniazid, only traces were found after pretreatment with pyrogallol. The normal increases in the concentrations of adrenaline and of isoprenaline-like activity in the lower aortic plasma of these preparations during pressor responses to intravenous adrenaline were also modified by pretreatment with these drugs. Whereas the monoamine oxidase inhibitors, harmaline and iproniazid, caused moderate excess in the adrenaline concentrations and great excess in the concentrations of isoprenaline-like activity found during pressor responses to adrenaline, the opposite occurred after pretreatment with cocaine (Table 2). By contrast, pyrogallol, which inhibits the *O*-methyl transferase, accentuated the normal rise in the plasma concentration of adrenaline and prevented the increase in isoprenaline-like activity with which this rise is normally associated (Table 2).

*Effect of antiadrenaline drugs and pretreatment with reserpine on the concentrations of amines in aortic plasma.* Pretreatment of cats with the antiadrenaline drugs phentolamine and dibenamine increased the concentrations of adrenaline and of isoprenaline-like activity in the plasma of blood withdrawn from the lower aorta both at rest and during pressor responses to intravenous adrenaline (Table 2). Resting levels of adrenaline and isoprenaline-like activity were also raised in cats which had received intramuscular reserpine 36 hr previously and in which the concentrations of pressor amines in saline extracts of the aortae were less than ⅓ of those of aortae taken from control animals. In these reserpine-treated animals, the increases in the concentrations of isoprenaline-like activity

TABLE 2

THE EFFECTS OF PRETREATMENT OF CATS WITH VARIOUS DRUGS ON THE CONCENTRATIONS OF ISOPRENALINE-LIKE SUBSTANCE APPEARING IN THE PLASMA

Blood samples were withdrawn from the lower aortae during pressor responses to intravenous injection of 4–8 μg/kg adrenaline. Experimental conditions as in Table 1

| Pretreatment | No. of cats | Plasma concentrations μg/100 ml. Adrenaline Resting | Adrenaline Responding | Isoprenaline Resting | Isoprenaline Responding |
|---|---|---|---|---|---|
| 1. None | (5) | 0·07±0·01 | 1·1±0·25 | <0·005 | 0·64±0·20 |
| 2. Harmaline | (3) | 1·05±0·06 | 2·3±0·50 | 0·27±0·20 | 8·40±3·60 |
| 3. Iproniazid | (2) | 0·14±0·05 | 3·8±0·20 | 3·51±0·86 | 8·04±2·37 |
| 4. Cocaine | (3) | 0·52±0·24 | 8·4±1·88 | 0·04±0·01 | 1·08±0·36 |
| 5. Pyrogallol | (2) | 1·20±0·80 | 5·0±2·41 | <0·03 | <0·03 |
| 6. Phentolamine | (3) | 0·44±0·01 | 3·6±0·47 | 0·06±0·01 | 2·10±0·80 |
| 7. Dibenamine | (3) | 0·62±0·04 | 5·7±0·36 | 0·05±0·02 | 1·72±0·27 |
| 8. Reserpine | (3) | 0·17±0·12 | 2·3±0·24 | 0·33±0·30 | 1·06±0·04 |

of the aortic plasma during pressor responses to adrenaline were not subnormal (Table 2).

*A search for a major site of origin of the isoprenaline-like activity.* Sites of origin of the isoprenaline-like activity were sought by measurement of change in its concentration in the plasma of venous blood from an organ or limb in response to injection of adrenaline into corresponding arteries. Whereas no isoprenaline-like activity could be detected in venous blood returning from a leg when adrenaline had been injected into the corresponding common iliac artery (Table 3), it appeared in the plasma of blood withdrawn from the inferior vena

TABLE 3

SITES OF ORIGIN OF THE ISOPRENALINE-LIKE COMPOUND

Changes in the concentration of isoprenaline-like activity in venous plasma in response to arterial injections of adrenaline 4–6 μg/kg. Experimental conditions as in Table 1

| Site of injection of adrenaline | Site of plasma sampling | μg/Isoprenaline/100 ml. plasma Resting | Responding |
|---|---|---|---|
| Abdominal aorta | Iliac vein | <0·005 (2) | <0·005 (2) |
| Splenic artery | Thoracic cava just above entry of hepatic veins | 1·54±0·02 (2) | 2·75±0·18 (2) |
| Hepatic artery | Thoracic cava just above entry of hepatic veins | 0·67±0·50 (3) | 3·40±0·02 (3) |

cava just above the entry of the hepatic veins in response to injections of adrenaline made either into the splenic or the hepatic arteries (Table 3). This indicated that the liver might function as a main source of isoprenaline-like activity. Comparison was therefore made of the concentrations of isoprenaline-like activity in plasma similarly withdrawn from the inferior vena cava at rest in response to the infusion of 5 μg adrenaline in 1 min either into the hepatic artery or into the portal vein. The mean concentrations of isoprenaline-like activity, expressed as μg isoprenaline/100 ml. plasma and followed by the standard errors of the means, found in three experiments were 0.05±0.01 at rest, and 1.6±0.26 and 4.3±1.31 as a result of the infusions into the hepatic artery and portal vein respectively. Finally, the effect of stimulation of hepatic nerves on the release of

isoprenaline-like activity was examined. Changes in the concentrations of this activity in plasma from blood taken from the inferior vena cava just above the entry of the hepatic veins were again measured, but in response to stimulation of the hepatic nerves, to injections of adrenaline into a femoral vein, and to both procedures effected simultaneously. The results of the three experiments made are shown in Table 4. Stimulation of the hepatic nerves was alone sufficient to increase the concentration of isoprenaline-like activity in the caval plasma, and the changes induced by stimulation summed with those of intravenous adrenaline.

TABLE 4

EFFECT OF STIMULATION OF THE HEPATIC NERVES ON THE CONCENTRATION OF ISOPRENALINE-LIKE ACTIVITY

Samples of thoracic caval blood were collected during, and in the absence of, intravenous injection of 10 $\mu$g adrenaline. Experimental conditions as in Table 1

| $\mu$g. Isoprenaline activity/100 ml. plasma | | | | No. of |
|---|---|---|---|---|
| Resting | Stimulation (a) | Adrenaline (b) | (a)+(b) | expts. |
| 0·035±0·06 | 0·64±0·16 | 2·67±1·58 | 3·85±0·81 | 3 |

## DISCUSSION

The isoprenaline-like activity (Fig. 1) found in the plasma of blood withdrawn from the lower abdominal aortae of cats during pressor responses to adrenaline might have been released by the adrenaline from preformed stores or have been due to the formation of a metabolite of adrenaline which closely resembled isoprenaline in $R_F$ value and pharmacological effect.

The first hypothesis is made unlikely by the following facts: first, no such stores of isoprenaline-like activity have been found in the lungs or blood vessels of cats (Lockett, unpublished observations). Secondly, the mechanism of the release from stores might be expected to be contractile or chemical. Yet noradrenaline, which closely resembles adrenaline both in contractile activity and in chemical structure, does not cause the appearance of an isoprenaline-like substance (Table 1), and block of the contractile effects of both adrenaline and noradrenaline with the antiadrenaline drugs phentolamine and dibenamine does not prevent the appearance of isoprenaline-like activity in response to the intravenous injection of adrenaline (Table 2).

The experimental results, however, support the view that the isoprenaline-like compound is a metabolite of adrenaline. In general, pretreatment with drugs which caused increase in the concentration of adrenaline in plasma withdrawn from the lower aorta during rest and supranormal increase in this concentration when adrenaline was injected intravenously also raised the resting and " responding " concentrations of isoprenaline-like activity in the plasma (Table 2). This was so, irrespective of the mechanism which occasioned the raised concentration of adrenaline in plasma except after pretreatment with pyrogallol, a compound which inhibits the *O*-methyl transferase (La Brosse, Axelrod & Kety, 1958 ; Bacq, Brown & Ferry, 1960). This last observation, coupled with the fact that the concentrations of isoprenaline-like substance were highest after pretreatment with the monoamine oxidase inhibitors harmaline and iproniazid (Udenfriend, Witkop,

Redfield & Weissbach, 1958), suggests that the *O*-methyl transferase system plays an essential part in the formation of the isoprenaline-like compound which, once formed, is destroyed by the monoamine oxidase. The fact that the plasma levels of the isoprenaline-like activity were raised disproportionately to those of adrenaline after pretreatment with reserpine may perhaps have resulted from inhibition of the absorption of adrenaline by blood cells (Hughes & Brodie, 1959) so that a greater proportion of the injected adrenaline became subjected to the action of the *O*-methyl transferase of liver and other tissues.

Not all tissues are capable of converting adrenaline to the isoprenaline-like compound (Table 3). The evidence indicates that the liver is a major site of its formation (Table 4) and the finding that stimulation of the hepatic nerves is alone sufficient to cause the appearance of isoprenaline-like activity in blood withdrawn from the thoracic portion of the inferior vena cava is consistent with previous observations (Lockett, 1957).

Several further points of interest arise from our experiments. The raised plasma levels both of adrenaline and isoprenaline-like compound found after pretreatment with reserpine support the view that reserpine not only liberates sympathetic amines from tissue stores (Bertler, Carlsson & Rosengren, 1956; Burn & Rand, 1958) but also prevents their absorption on blood cells (Hughes & Brodie, 1959). Similarly raised plasma levels of adrenaline after pretreatment with harmaline and iproniazid suggest circulating adrenaline is in part inactivated by the monoamine oxidase without prior *O*-methylation, or that the accumulation of *O*-methylated adrenaline resulting from inhibition of the monoamine oxidase slows activity of the transferase. The increased plasma concentrations of adrenaline after phentolamine and dibenamine in arterial blood supplement the observations of Brown & Gillespie (1957) and Brown, Davies & Gillespie (1958) made on venous blood. These authors suggested that the increase in the concentrations of amines in the venous blood organs during stimulation of the sympathetic supply which followed the use of antiadrenaline drugs might result from the absence of their utilization at receptor sites. More work on the mode of action of these drugs is needed before this sole explanation can be accepted with confidence.

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