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THE MOBILIZATION OF FREE FATTY ACIDS IN RESPONSE TO ISOPRENALINE IN THE RAT

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The plasma concentration of albumin-bound free fatty acids is increased by the administration of adrenaline or noradrenaline to experimental animals (Havel & Goldfien, 1959; Schotz & Page, 1959). Similarly, incubation of adipose tissue *in vitro* with either catechol amine leads to both an accumulation of free fatty acids within the tissue and an increased concentration in the medium in the presence of albumin as an acceptor (Gordon & Cherkes, 1958; White & Engel, 1958). These changes following adrenaline have been held to represent mobilization of depot fat in the readily transportable form of albumin-bound free fatty acids (Dole, 1956; Gordon & Cherkes, 1956; Gordon, 1957). Further evidence for the implication of the sympathetic nervous system in the mobilization of lipid comes from the discovery of significant quantities of noradrenaline in adipose tissue (Paoletti, Smith, Maickel & Brodie, 1961). In addition, stimuli associated with increased sympathetic nervous activity such as fear (Cardon & Gordon, 1959), psychic stress (Bogdonoff, Estes & Trout, 1959), hypoxia (McElroy & Spitzer, 1961) and cigarette smoking (Kershbaum, Bellet, Dickstein & Feinberg, 1961) all induce elevated plasma levels of free fatty acids. It has been suggested that the lipolytic effects of sympathomimetic amines are not mediated by the stimulation purely of α- or purely of β-receptors, nor by combined stimulation of both receptor types (Wenke, Mühlbachová & Hynie, 1961). Before testing this hypothesis further it seemed advisable to examine the effects of isoprenaline on fat mobilization in similar detail to the existing studies on adrenaline and noradrenaline. Reports in the literature indicate that intravenous infusions of isoprenaline in man produce marked increases in plasma free fatty acid levels (Bruce, Cobb & Williams, 1961; Mueller & Horwitz, 1962). Similar results have been obtained in anaesthetized dogs (Mayer, Moran & Fain, 1961; Ashmore, Preston & Love, 1962). In the rat, an increase in the content of free fatty acid in the epididymal fat body after parenteral isoprenaline has been described and the compound also increased the release of free fatty acid from adipose tissue *in vitro* (Wenke *et al.*, 1961).

METHODS

The rats used were specific pathogen-free males of the Alderley Park (albino) strain. They were fed on a cubed diet and water supplied *ad libitum*. The rats were housed in groups of five in a room maintained at 21° C in the absence of other laboratory species. The weight of rats used for experiments *in vivo* was 190 to 210 g whereas donors of adipose tissue for work *in vitro* weighed 140 to 150 g. All experiments were conducted between 9.00 a.m. and 1.00 p.m. in the animal house itself. The animal attendant was not allowed in the room until after the experiments had been completed and precautions were taken to avoid unnecessary noise or talking.

Intravenous injections, in volumes of 0.2 ml./100 g body weight, were made into an exposed saphenous vein whilst the animal was under light ether anaesthesia. At appropriate time intervals the rats were again anaesthetized with ether and blood samples withdrawn from the abdominal aorta into a heparinized syringe.

Intravenous infusions, at the rate of 0.03 ml./100 g/min were made into an exposed saphenous vein of rats anaesthetized 15 min previously with pentobarbitone (60 mg/kg, intraperitoneally). Blood samples were withdrawn from the abdominal aorta at appropriate time intervals. Control rats were bled 15 min after pentobarbitone without infusion.

Blood pressure was recorded by means of a Condon mercury manometer from a carotid artery of rats anaesthetized with pentobarbitone.

Studies *in vitro* were carried out as follows. Epididymal fat pads were removed during pentobarbitone anaesthesia from rats which had been fasted overnight. The pads were cut into two approximately equal halves which were blotted and weighed on a torsion balance. Each segment was then placed in 2.9 ml. of Krebs-Ringer-bicarbonate solution containing 3% bovine serum albumin in a 10-ml. conical flask. Catechol amines, dissolved in distilled water, were prepared in concentrations such that the addition of 0.1 ml. per flask yielded the desired final concentration. One segment from each animal received a control addition of distilled water only. Different concentrations were made in random order of flasks, which were incubated at 37° C for 3 hr in a Dubnoff Metabolic Shaker. At the end of the incubation period, a 1-ml. aliquot was removed for free fatty acid analysis. The results were calculated as micro-equivalents (μequiv) of fatty acid (in terms of palmitic acid) released per g of fat per hr of incubation. The response to a given concentration of catechol amine was taken as the difference between stimulated segments and the control segment from the same rat.

The free fatty acid content of plasma or incubates was determined by the method of Dole (1956) as modified by Barrett (1964). Blood glucose was estimated by a glucose oxidase technique and plasma corticosterone as described by Barrett & Stockham (1963).

The drugs used were: (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate, (±)-isoprenaline sulphate, (−)-isoprenaline acid tartrate, 5-hydroxytryptamine creatinine sulphate, acetylcholine chloride and histamine acid phosphate. All concentrations have been expressed in terms of base per unit volume. For experiments *in vivo* drugs were dissolved in 0.9% saline, and for studies *in vitro* distilled water was used.

RESULTS

The exposure of rats to ether vapour, sufficient to induce general anaesthesia for about 1 min, was followed by a rise in the concentration of plasma free fatty acids. If, during anaesthesia, the skin overlying the saphenous vein was cut and an intravenous injection of 0.9% saline was made, there was little change in the pattern of response. From the results summarized in Table 1, it can be seen that the inclusion of 10 μg/kg of adrenaline in the injection added little to the response to ether alone. When the adrenaline was increased to 20 μg/kg a significant increase in plasma free fatty acids, over and above the response to saline, occurred both 5 and 10 min after injection. Increasing the dose to 40 μg/kg resulted in the death of all the treated animals (from pulmonary oedema). Essentially similar responses at the 20-μg/kg level were seen with adrenaline, noradrenaline and 5-hydroxytryptamine. However, the response to the same dose of isoprenaline was much greater and, in addition, the peak fatty acid level was reached 5 min after injection whereas with the other groups the maximum occurred at 10 min. There was no significant difference between the mean values at 10 min for any of the 20-μg/kg dosed groups.

A more careful examination of the time-relationships of the fatty acid changes after isoprenaline administration confirmed that the maximal response was at 5 min. The response to doses in the range of 5 to 80 μg/kg are shown in Table 2.

The concentration of plasma free fatty acids was approximately doubled by 40 μg/kg

TABLE 1

PLASMA FREE FATTY ACID CONCENTRATIONS IN RATS BEFORE AND AFTER VARIOUS PROCEDURES

Values are means and standard errors. The numbers in parentheses indicate the numbers of animals contributing to the means. * Significant difference ($P<0.05$) from the zero time control. † Significant difference ($P<0.05$) from the saline control at corresponding time. All injections were intravenous

Procedure	Plasma free fatty acid (μequiv/l.) at time after procedure			
	0 min	5 min	10 min	20 min
Ether anaesthesia alone (60 sec)	393±17 (7)	431±42 (7)	526±46 (6)*	465±38 (6)
Ether+saline (0·5 ml./100 g)	431±17 (9)	412±16 (8)	513±26 (7)*	483±25 (9)
Ether+adrenaline (10 μg/kg)	404±15 (4)	501±52 (4)	528±35 (4)*	432±29 (4)
Ether+adrenaline (20 μg/kg)	437±18 (17)	505±25 (10)*†	707±20 (10)*†	611±60 (4)*
Ether+noradrenaline (20 μg/kg)	434±16 (19)	602±44 (13)*†	623±37 (10)*†	583±36 (4)*
Ether+isoprenaline (20 μg/kg)	422±18 (13)	842±72 (8)*†	756±37 (6)*†	555±12 (6)*
Ether+5-hydroxy-tryptamine (20 μg/kg)	429±11 (5)	492±45 (5)	744±48 (5)*†	553±35 (5)*

TABLE 2

PLASMA FREE FATTY ACID CONCENTRATIONS IN RATS BEFORE AND 5 MIN AFTER VARIOUS DOSES OF ISOPRENALINE OR OF SALINE (0·5 ML./100 G)

There were six animals in each group. Values are means and standard errors

Dose (μg/kg)	Plasma free fatty acids (μequiv/l.)	
	Concentration	Increment
0	400±21	—
5	435±24	35±32
10	561±50	161±54
20	741±25	341±33
40	809±29	409±36
80	837±32	437±38
Saline	424±39	24±44

but no significant additional increase was seen at 80 μg/kg. The increment for each dose-level was calculated as the difference between the uninjected controls and the dose-injected group. When the increments were plotted against the dose of isoprenaline injected a typical sigmoid dose/response curve was obtained.

The increment in plasma free fatty acids appeared to be independent of the initial concentration or the nutritional status of the animals. Two groups of six rats were fasted for 24 hr and the fatty acid level was determined in one group. The other group received an intravenous injection of 20 μg/kg of isoprenaline and was bled 5 min later. The control fasting level was 875±78 μequiv/l. as compared with 1,197±110 μequiv/l. in the injected group. The increment, 322±135 μequiv/l., was not significantly different from that found in fed rats (341±33 μequiv/l.).

Because of the lethality of higher doses of adrenaline and noradrenaline it was not possible to compare their activities as mobilizers of fatty acids with that of isoprenaline. Furthermore, single intravenous injections do not always lead to identical blood levels of the compound injected, since the rate of injection is not always the same. It was decided,

TABLE 4

INCREMENTS IN PLASMA FREE FATTY ACID CONCENTRATIONS AT THE END OF A 15-MIN INTRAVENOUS INFUSION OF VARIOUS DOSES OF CATECHOL AMINES IN RATS ANAESTHETIZED WITH PENTOBARBITONE

The numbers in parentheses indicate the numbers of animals contributing to the means. Values are means and standard errors. Saline (0·5 ml./100 g over 15 min) was given to the rats that had no catechol amine

	Increment in free fatty acids (μequiv/l.) for infusion rate (μg/kg/min)						
Catechol amine	0·0	0·2	0·4	0·8	1·6	3·2	6·4
None	18±16 (11)	—	—	—	—	—	
Adrenaline	—	—	—	226±62 (7)	357±32 (5)	467± 37 (9)	582±49 (3)
(−)-Noradrenaline	—	—	—	265±40 (9)	469±78 (7)	667± 64 (7)	674±93 (7)
(±)-Isoprenaline	—	—	137±57 (6)	403±56 (9)	615±36 (5)	664±102 (6)	641±63 (7)
(−)-Isoprenaline	—	118±36 (12)	402±83 (12)	572±51 (8)	621±66 (8)	—	—

therefore, to compare the effects of intravenous infusions of adrenaline, noradrenaline and isoprenaline in animals anaesthetized with pentobarbitone. In preliminary experiments, it was found that the plasma level of free fatty acids fell in rats treated with pentobarbitone if the level was initially high due to excitement or noise. However, in rats housed in conditions of quiet, there was no significant change in the free fatty acid concentration during the first hour of anaesthesia. Pentobarbitone itself did not depress the rate of release of free fatty acids in response to isoprenaline, since the 5-min increment following a 20-μg/kg dose was the same in both pentobarbitone-treated rats or those injected during ether anaesthesia.

The first infusions were arranged to allow the administration of 20 μg/kg over 25 min. Infusion of 0.9% saline alone had no significant effect on the concentration of plasma free fatty acids. Subsequently, results were expressed as the difference in free fatty acids between anaesthetized uninfused control rats on the day of experiment and animals infused with catechol amines. The mean changes at different time intervals after the infusion of catechol amines are presented in Table 3.

TABLE 3

INCREMENTS IN PLASMA FREE FATTY ACID CONCENTRATIONS AT VARIOUS TIMES DURING THE INTRAVENOUS INFUSION OF CATECHOL AMINES INTO RATS ANAESTHETIZED WITH PENTOBARBITONE

The infusion rate was 0·8 μg/kg/min. Values are means and standard errors. The numbers in parentheses indicate the numbers of animals contributing to the means. Saline (0·3 ml./kg/min) was given to the rats that had no catechol amine

Catechol amine	Increment in free fatty acids (μequiv/l.) at time of infusion (min)			
	5	10	15	25
None	1±17 (4)	19±21 (4)	12±17 (4)	29±41 (4)
(−)-Adrenaline	−100±27 (5)	140±40 (9)	226±62 (7)	269±60 (8)
(−)-Noradrenaline	86±67 (5)	154±41 (9)	265±40 (9)	330±69 (6)
(±)-Isoprenaline	344±18 (5)	487±38 (11)	403±56 (9)	508±71 (6)

Again, the greater activity of isoprenaline was demonstrated as was the earlier rise to maximal values. The responses to adrenaline and noradrenaline were similar except at the 5-min interval when adrenaline induced a fall of about 30% quite consistently. Although the 25-min values were numerically higher than those observed at 15 min, there was no statistically significant difference between the two levels for any one amine.

Adopting a 15-min period of infusion as standard, various concentrations of catechol amine were compared to establish dose/response curves. The results, summarized in Table 4, showed that the maximum increment was between 600 and 700 μequiv/l. Since the control values ranged between 290 and 350 μequiv/l. this represented an increase of 200% or an absolute value in the region of 1,000 μequiv/l. The greater activity of isoprenaline was clearly shown even when the racemate was compared with the pure laevo-forms of adrenaline and noradrenaline. Pure (−)-isoprenaline possessed about twice the activity of the racemate indicating that the dextro-form has relatively little activity in this system. When all the results were expressed as a percentage of the response to noradrenaline at 6.4 μg/kg/min (674±93 μequiv/l.) and plotted against dose, it was possible to estimate roughly the activities at the 50% effect level. Taking the three laevo-forms only, the rates were: isoprenaline—noradrenaline—adrenaline: 0.34—1.03—1.64 μg/kg/min to produce 50% of the maximum increase in plasma free fatty acids. It was apparent that the curves for (−)-isoprenaline,

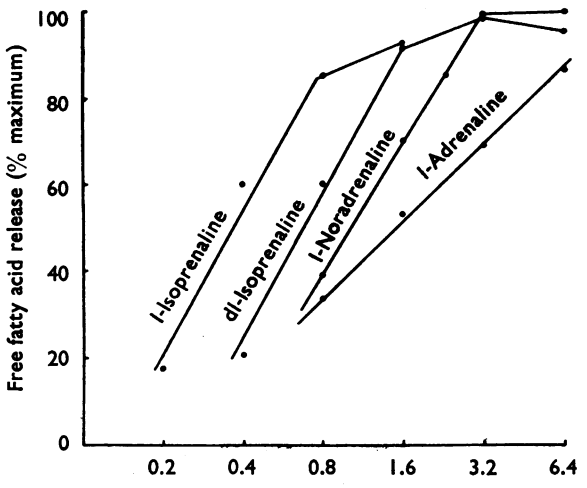

Fig. 1. Increments in plasma concentration of free fatty acids after 15 min of infusion of catechol amines at different dose levels (log scale). Values are expressed as percentages of maximum response, calculated from the values in Table 4.

(±)-isoprenaline and noradrenaline were almost parallel. The adrenaline response curve was, however, less steep. Since adrenaline is, of the substances tested, the most powerful in causing hyperglycaemia, and since glucose is known to decrease free fatty acid release, it was decided to determine the changes in blood sugar level concomitantly with that of free fatty acids after infusion of the three catechol amines.

The results of such an experiment are summarized in Table 5. In addition to the free fatty acid and blood sugar levels, plasma corticosterone concentration was also determined since catechol amines are known to release pituitary corticotrophin which itself is capable of mobilizing fat. At the dose level chosen (0.8 μg/kg/min) the free fatty acid responses to adrenaline and noradrenaline were very similar but, whereas the blood sugar level almost doubled after adrenaline, the 9% increase after noradrenaline was only just statistically significant ($P<0.05$). In contrast isoprenaline had no effect on the blood sugar level whilst having a considerably greater action than adrenaline and noradrenaline upon the plasma

TABLE 5

THE CONCENTRATION OF PLASMA FREE FATTY ACIDS AND CORTICOSTERONE AND THE BLOOD SUGAR LEVEL OF RATS BEFORE AND AFTER A 15-MIN INFUSION OF CATECHOL AMINES (0·8 μG/KG/MIN)

Preinfusion refers to 15 min after pentobarbitone. Saline (0·3 ml./kg/min) was given to the rats that had no catechol amine

Treatment	No. of rats	Free fatty acids (μequiv/l.)	Blood sugar (mg/100 ml.)	Corticosterone (μg/100 ml.)
Preinfusion	6	289±24	90±2·5	11·9±2·1
None	6	287±20	89±2·1	40·2±1·1
Adrenaline	6	509±31	174±3·1	42·2±1·7
Noradrenaline	6	516±29	98±1·4	41·4±0·9
(±)-Isoprenaline	6	816±41	85±4·8	40·5±1·7

TABLE 6

THE INCREASE IN FREE FATTY ACID RELEASE FROM RAT EPIDIDYMAL ADIPOSE TISSUE INCUBATED *IN VITRO* WITH VARIOUS CONCENTRATIONS OF ADRENALINE, NORADRENALINE AND ISOPRENALINE

Each value represents the mean (± standard error) of the difference between control fat segments incubated with distilled water and test segments to which catechol amine had been added. The figures in parentheses indicate the number of segments incubated. The mean value for control segments was 3·52±0·17 (49) μequiv/g/hr

Concentration of amine (μg/ml.)	Catechol amine added		
	(−)-Adrenaline	(−)-Noradrenaline	(±)-Isoprenaline
0·05	0·86±0·30 (5)	1·70±0·54 (5)	4·07±0·57 (5)
0·10	0·81±0·45 (7)	1·86±0·40 (5)	5·69±0·34 (11)
0·20	2·35±0·34 (8)	3·54±0·58 (5)	7·35±0·48 (11)
0·40	6·70±0·52 (23)	7·53±0·63 (17)	10·22±0·55 (8)
0·80	10·29±1·18 (5)	11·50±1·53 (5)	13·47±0·86 (5)
1·60	10·21±0·67 (7)	12·20±0·80 (7)	13·16±0·90 (7)

concentration of free fatty acids. In all the infused groups the plasma corticosterone level had risen to approximately 40 μg/100 ml. which indicated that the technique of infusion was itself causing a maximal release of corticotrophin. Since the changes in plasma free fatty acids showed no correlation with plasma corticosterone or blood sugar levels it was considered likely that the fat mobilizing activities of the three catechol amines were intrinsic properties of the individual molecules.

The ratio of activities was also confirmed in an *in vitro* system in the absence of glucose. The results of incubations of epididymal fat pads with various concentrations of adrenaline, noradrenaline and isoprenaline are summarized in Table 6. By converting each response to a percentage of the maximum for each amine, the following estimates are obtained of the concentration required to increase fatty acids released to 50% of the maximum, together with the 95% limits: (±)-isoprenaline 1.70×10^{-7} (0.10–0.28); (−)-noradrenaline 2.57×10^{-7} (0.16–0.41); and (−)-adrenaline 3.15×10^{-7} (0.21–0.48). Unfortunately, (−)-isoprenaline was not available to the author at the time of experiment and when it was eventually compared with the racemate the sensitivity of the *in vitro* system had decreased. Regardless of this fact, the laevo-form was found to possess twice the activity of the racemate over a range of concentrations. If one makes the assumption that the 50% effective concentration of (−)-isoprenaline in the original *in vitro* would have been 0.85×10^{-7} the activities *in vivo* and *in vitro* can be compared (Table 7). There is excellent agreement for the ratios of noradrenaline and isoprenaline but a discrepancy for adrenaline. The higher figure in the *in vivo* system is most probably due to a depressant action of an increased blood sugar level.

TABLE 7

COMPARISON OF THE ACTIVITIES FOR (−)-ADRENALINE, (−)-NORADRENALINE, (±)-ISOPRENALINE AND (−)-ISOPRENALINE ON THE MOBILIZATION OF FREE FATTY ACIDS AFTER INFUSION *IN VIVO* OR INCUBATION WITH FAT PADS *IN VITRO*

Catechol amine	*In vivo*		*In vitro*	
	ED50 (μg/kg/min)	Ratio	ED50 (μg/ml.)	Ratio
(−)-Isoprenaline	0·34	1·00	(0·085)	1·00
(±)-Isoprenaline	0·70	0·49	0·170	0·50
(−)-Noradrenaline	1·03	0·33	0·257	0·33
(−)-Adrenaline	1·64	0·21	0·315	0·27

Rapid inactivation of catechol amines is likely to occur when they are incubated at 37° C at neutral *p*H even without the addition of adipose tissue. No pharmacological activity was found in solutions containing 10 μg/ml. after 30 min incubation. Since the concentrations studied were much smaller in the foregoing experiments, inactivation was probably more rapid and it was just possible that differences in the rate of inactivation might account for the greater activity of isoprenaline. However, the addition of ascorbic acid to the medium did not influence the relative activities although it increased the sensitivity of the system by a factor of ten.

The overall effect of isoprenaline upon the blood pressure of the anaesthetized rat is hypotensive whereas both adrenaline and noradrenaline have pressor action. It was possible that the vasodilatation following isoprenaline infusion led to a redistribution of blood in favour of fat-liberating areas. The infusion of acetylcholine at 0.8 μg/kg/min resulted in a fall in blood pressure similar to that seen after isoprenaline at the same dose level. However, acetylcholine only caused a slight rise in plasma free fatty acids in comparison with isoprenaline (Table 8).

TABLE 8

PLASMA CONCENTRATIONS OF FREE FATTY ACIDS AND CORTICOSTERONE BEFORE AND AFTER A 15-MIN INTRAVENOUS INFUSION OF ISOPRENALINE, ACETYLCHOLINE OR HISTAMINE AT VARIOUS DOSE LEVELS

* Significant difference ($P<0.05$) from preinfusion levels

Drug	Infusion rate (μg/kg/min)	No. of rats	Free fatty acids (μequiv/l.)	Corticosterone (μg/100 ml.)
None	—	4	308±27	8·9±0·9
(±)-Isoprenaline	0·8	4	711±102*	25·4±3·1*
Acetylcholine	0·8	4	396±19*	32·4±7·1*
	1·6	4	345±15	—
	3·2	4	369±19	—
Histamine	333	4	318±31	35·1±6·4*

When the dose of acetylcholine was increased there was no corresponding increase in fatty acid release. Histamine, at the dose given, again produced a fall in mean arterial pressure similar to that produced by isoprenaline at 0.8 μg/kg/min but was devoid of action on plasma concentration of free fatty acids. Plasma corticosterone determinations were performed in this experiment because of the well-known corticotrophin-releasing effect of histamine. The failure of histamine infusion to stimulate any change in the plasma level of free fatty acids indicates that, in the rat, acute release of physiological amounts of corticotrophin has little influence over the rate of free fatty acid mobilization.

DISCUSSION

The concentration of free fatty acids in the plasma of rats is extremely sensitive to many adventitious factors (Barrett, 1964). This makes it difficult to compare the free fatty acid mobilizing activity of a series of compounds in a quantitative manner. Although ether anaesthesia is a convenient technique for the immobilization of rats for the withdrawal of blood samples without significant change in plasma fatty acids, recovery from anaesthesia is invariably accompanied by a rise in the acid titre. Similar rises in the level of fatty acids follow restraint before intravenous injection. Presumably the rise in both instances is mediated by endogenous catechol amine release. Intravenous infusions have many

advantages over injections but are not easy to arrange in undisturbed conscious rats. The requirement is for an immobilizing agent which will suppress endogenous changes in plasma free fatty acids without affecting their responsiveness to administered drugs. The present experiments suggest that pentobarbitone anaesthesia is a suitable technique for this purpose.

All three catechol amines produced highly significant increases in the concentration of free fatty acids when injected or infused intravenously and stimulated the release of fatty acids from isolated adipose tissue incubated *in vitro*. In each experimental situation isoprenaline was the most active amine, followed by noradrenaline with adrenaline the least active. The order of activity for fat mobilization is the reverse of that given for hyperglycaemic action (Ellis, 1956). There is considerable conflict in the literature concerning the classification of the hyperglycaemic response and it would appear that neither this nor the fat mobilizing effect of the catechol amines can be defined as exactly as the cardiovascular responses in terms of receptor types.

Noradrenaline has been found about twice as active as adrenaline in increasing the plasma level of free fatty acids in the dog (Mayer *et al.*, 1961; Spitzer & Gold, 1962). The lesser effect of adrenaline was attributed to its concomitant hyperglycaemic effect by Mayer *et al.* (1961). It is well known that the blood-levels of glucose and free fatty acids are related reciprocally under certain circumstances. During fasting the fatty acid level rises whilst glucose concentration is low but an infusion of glucose brings about a prompt fall in fatty acid concentrations (Gordon & Cherkes, 1956). It might be argued, therefore, that the fat-mobilizing activity of isoprenaline was greatest because its hyperglycaemic effect was the weakest. However, since adrenaline was also found the least active releaser of free fatty acids *in vitro*, it is unlikely that the differences in activity can be accounted for solely in terms of hyperglycaemic effect. Further, isoprenaline produced the same increment in plasma free fatty acids in both fed and fasting rats, while it is known that isoprenaline is only hyperglycaemic in the fasting state (Fleming & Kenny, 1964). Also, equipressor doses of adrenaline and α-methylnoradrenaline have similar effects on the blood sugar but the latter agent has a considerably greater effect on plasma fatty acids (Mueller & Horwitz, 1962). It is doubtful therefore that combined hyperglycaemic and fat-mobilizing activity in the same molecule necessarily renders the substance less effective on fatty acid release than a molecule without hyperglycaemic effects.

The shapes of the time relationship curves for adrenaline were markedly different from those for noradrenaline and isoprenaline. Whereas noradrenaline or isoprenaline infusions always produced a progressive increase in plasma free fatty acids there was always an initial fall in their plasma concentration with the infusion of adrenaline ($P<0.05$). A similar initial decrease has been observed in the dog (Shafrir & Steinberg, 1960) when it was considered to reflect an increased rate of utilization of free fatty acids, temporarily exceeding the rate of fatty acid release from the fat depots. Bruce *et al.* (1961) have suggested that isoprenaline stimulates the release of free fatty acids without increasing their uptake, since they found that there was a similar increase in arterial and venous levels after isoprenaline administration. There is much evidence supporting the view that fatty acid uptake is directly proportional to the circulating concentration (Steinberg, 1964). However, it is not known whether or not the three catechol amines studied here possess differing stimulating effects on the uptake of free fatty acids independent of their releasing activity.

There is considerable interest in the mechanism of the calorigenic action of catechol

amines. For noradrenaline, evidence has been presented in support of a causal relationship between its calorigenic effect and its ability to increase the rate of free fatty acid mobilization (Steinberg, Nestel, Buskirk & Thompson, 1964). On the other hand, Lundholm & Svedmyr (1964) consider that calorigenesis is more closely related to the production and oxidation of lactic acid. In studies on the rabbit, they found the relative activities of isoprenaline, adrenaline and noradrenaline to be 1 : 0.11 : 0.008 in respect both of stimulation of oxygen consumption and of lactic acid production. Comparative data on fat-mobilizing and calorigenic activity of catechol amines in the rat are not yet available but preliminary studies in this laboratory suggest that calorigenic activity follows fat mobilizing rather than lactic acid producing activity in this species. (Despite the findings of Trout, Estes & Friedberg (1960) concerning the interference by lactic acid in the method of Dole (1956), physiological levels of lactic acid have not been found to alter free fatty acid titration values in this laboratory. It is unlikely, therefore, that the observed differences in catechol amine potency could be due to different degrees of lactic acid contamination.)

It has been suggested (Rudman, 1965) that part, if not all, of the fat-mobilizing activity of catechol amines *in vivo* may be mediated by the release of pituitary corticotrophin. The evidence for catechol amine-induced corticotrophin release has been reviewed by Harris (1955). Studies of the comparative activity of adrenaline, noradrenaline and isoprenaline have shown that isoprenaline is the most and noradrenaline the least active in this context (Nasmyth, 1950; Jarrett, 1951). The difference in activity of these sympathomimetic drugs on free fatty acid release *in vivo* might therefore be related to their corticotrophin-releasing potential. In this study, however, maximal plasma corticosteroid values were found after the infusion of 0.9% saline, histamine or catechol amines, yet neither saline nor histamine evoked a significant change in plasma fatty acids. Furthermore, Paoletti *et al.* (1961) found that after catechol amine depletion by reserpine the lipolytic effects of corticotrophin *in vivo* were abolished. Many authors have noted the " exquisite sensitivity " of isolated adipose tissue to the fat-mobilizing effects of corticotrophin and other peptides of pituitary origin. The minimal effective concentration of corticotrophin is of the order of 0.1 μg/ml. (Engel, 1961) which is equivalent to 1.5 mU/ml. in terms of purified corticotrophin (Li, 1962). However, the circulating level of corticotrophin in severely stressed male rats is below the minimum detectable level which is 0.005 mU/ml. (Sydnor & Sayers, 1954; Barrett, 1959). The results described in this paper do not suggest any role for corticotrophin in catechol amine-induced release of free fatty acids and support the view of Friesen (1964) that fat mobilization by corticotrophin has not been shown to be more than a pharmacological effect.

SUMMARY

1. The effects of intravenous injections and infusions of adrenaline, noradrenaline and isoprenaline on the plasma level of free fatty acids were studied in rats. The three catechol amines were also compared in an isolated adipose tissue system *in vitro*.

2. Infusion of amines in rats anaesthetized with pentobarbitone was found to be a satisfactory method for quantitative comparison. The infusion of freshly prepared 0.9% saline in glass-distilled water had no significant effect on plasma free fatty acid levels.

3. Isoprenaline was the most potent releasing agent of free fatty acids in all systems studied. The relative activities of isoprenaline, noradrenaline and adrenaline were 1 : 0.33 : 0.2.

4. Whereas adrenaline produced a significant fall in the concentration of free fatty acids before producing an increase, infusions of isoprenaline and noradrenaline always produced a progressive increase up to a maximum value. There was no significant difference in the maximum plasma free fatty acid level produced by each catechol amine.

5. The difference in potency was not directly attributable to differences in hyperglycaemic activity, cardiovascular response or release of pituitary corticotrophin.

6. The results do not support the existence of a simple β-receptor mechanism for catechol amine-induced release of free fatty acids in the rat.

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REFERENCES

ASHMORE, J., PRESTON, J. E. & LOVE, W. C. (1962). Effects of dichloroisoproterenol on blood sugar and free fatty acids. *Proc. Soc. exp. Biol. (N.Y.)*, **109**, 291–294.

BARRETT, A. M. (1959). *Some Observations on the Mechanisms Controlling the Release of Adrenocorticotrophin.* Ph.D. thesis at the University of London.

BARRETT, A. M. (1964). Adventitious factors affecting the concentration of free fatty acids in the plasma of rats. *Brit. J. Pharmacol.*, **22**, 577–584.

BARRETT, A. M. & STOCKHAM, M. A. (1963). The effect of housing conditions and simple experimental procedures upon the corticosterone level in the plasma of rats. *J. Endocr.*, **26**, 97–105.

BOGDONOFF, M. D., ESTES, E. H. & TROUT, D. (1959). Acute effect of psychologic stimuli upon plasma non-esterified fatty acid levels. *Proc. Soc. exp. Biol. (N.Y.)*, **100**, 503–504.

BRUCE, R. A., COBB, L. A. & WILLIAMS, R. H. (1961). Effects of exercise and isoproterenol on free fatty acids and carbohydrates in cardiac patients. *Amer. J. med. Sci.*, **241**, 59–67.

CARDON, P. V. & GORDON, R. S. (1959). Rapid increase of plasma unesterified fatty acids in man during fear. *J. psychosom. Res.*, **4**, 5–7.

DOLE, V. P. (1956). A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. clin. Invest.*, **35**, 150–154.

ELLIS, S. (1956). The metabolic effects of epinephrine and related amines. *Pharmacol. Rev.*, **8**, 486–562.

ENGEL, F. L. (1961). Extra-adrenal actions of adrenocorticotropin. *Vitam. and Horm.*, **19**, 189–227.

FLEMING, W. W. & KENNY, A. D. (1964). The effect of fasting on the hyperglycaemic responses to catechol amines in rats. *Brit. J. Pharmacol.*, **22**, 267–274.

FRIESEN, H. (1964). Pituitary peptides and fat mobilization. *Metabolism*, **13**, 1214–1229.

GORDON, R. S. (1957). Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty acid. *J. clin. Invest.*, **36**, 810–815.

GORDON, R. S. & CHERKES, A. (1956). Unesterified fatty acid in human blood plasma. *J. clin. Invest.*, **35**, 206–212.

GORDON, R. S. & CHERKES, A. (1958). Production of unesterified fatty acids from isolated rat adipose tissue incubated *in vitro*. *Proc. Soc. exp. Biol. (N.Y.)*, **97**, 150–151.

HARRIS, G. W. (1955). *Neural Control of the Pituitary Gland.* London: Arnold.

HAVEL, R. J. & GOLDFIEN, A. (1959). Role of the sympathetic nervous system in the metabolism of free fatty acids. *J. Lipid Res.*, **1**, 102–108.

JARRETT, I. G. (1951). The effect of adrenaline, noradrenaline and isopropylnoradrenaline on the ascorbic acid content of the rat's adrenal. *Brit. J. Pharmacol.*, **6**, 294–298.

KERSHBAUM, A., BELLET, S., DICKSTEIN, E. R. & FEINBERG, L. J. (1961). Effect of cigarette smoking and nicotine on serum free fatty acids. *Circulat. Res.*, **9**, 631–638.

LI, C. H. (1962). Synthesis and biological properties of ACTH peptides. *Recent Progr. Hormone Res.*, **18**, 1–40.

LUNDHOLM, L. & SVEDMYR, N. (1964). Comparative investigation of the calorigenic and lactic acid stimulating effects of isoprenaline and adrenaline in experiments on rabbits. *Acta physiol. scand.*, **62**, 60–67.

MAYER, S., MORAN, N. C. & FAIN, J. (1961). The effect of adrenergic blocking agents on some metabolic actions of catechol amines. *J. Pharmacol. exp. Ther.*, **134**, 18–27.

MCELROY, W. T. & SPITZER, J. J. (1961). Hypoxia as a stimulus for free fatty acid mobilization in dogs. *J. appl. Physiol.*, **16**, 760–762.

MUELLER, P. S. & HORWITZ, D. (1962). Plasma free fatty acid and blood glucose responses to analogues of norepinephrine in man. *J. Lipid Res.*, **3**, 251–255.

NASMYTH, P. A. (1950). The effect of some sympathomimetic amines on the ascorbic acid content of rats' adrenal glands. *J. Physiol. (Lond.)*, **110**, 294–300.

PAOLETTI, R., SMITH, R. L., MAICKEL, R. P. & BRODIE, B. B. (1961). Identification and physiological role of norepinephrine in adipose tissue. *Biochem. biophys. Res. Commun.*, **5**, 424–429.

RUDMAN, D. (1965). The fat mobilizing property of hypophyseal peptides and aromatic amines. *Ann. N.Y. Acad. Sci.*, in the press.

SCHOTZ, M. C. & PAGE, I. H. (1959). Effect of norepinephrine and epinephrine on non-esterified fatty acid concentration in plasma. *Proc. Soc. exp. Biol. (N.Y.)*, **101**, 624–626.

SHAFRIR, E. & STEINBERG, D. (1960). The essential role of the adrenal cortex in the response of plasma free fatty acids, cholesterol and phospholipids to epinephrine injection. *J. clin. Invest.*, **39**, 310–319.

SPITZER, J. J. & GOLD, M. (1962). Effect of catechol amines on the individual free fatty acids of plasma. *Proc. Soc. exp. Biol. (N.Y.)*, **110**, 645–647.

STEINBERG, D. (1964). The fate of plasma free fatty acids and their effect on tissue metabolism. *Metabolism*, **13**, 1264–1272.

STEINBERG, D., NESTEL, P. J., BUSKIRK, E. R. & THOMPSON, R. H. (1964). Calorigenic effect of norepinephrine correlated with plasma free fatty acid turnover and oxidation. *J. clin. Invest.*, **43**, 167–176.

SYDNOR, K. L. & SAYERS, G. (1954). Blood and pituitary ACTH in intact and adrenalectomised rats after stress. *Endocrinology*, **55**, 621–636.

TROUT, D. L., ESTES, E. H. & FRIEDBERG, S. J. (1960). Titration of free fatty acids of plasma: a study of current methods and a new modification. *J. Lipid Res.*, **1**, 199–202.

WENKE, M., MÜHLBACHOVÁ, E. & HYNIE, S. (1961). The actions of adrenotropic substances on depot lipids. *Biochem. Pharmacol.*, **8**, 140–141.

WHITE, J. E. & ENGEL, F. L. (1958). A lipolytic action of epinephrine and norepinephrine on rat adipose tissue *in vitro*. *Proc. Soc. exp. Biol. (N.Y.)*, **99**, 375–378.