Brit. J. Pharmacol. (1954), 9, 498.

IDENTIFICATION OF AN ISOPRENALINE-LIKE SUBSTANCE IN EXTRACTS OF ADRENAL GLANDS

BY

MARY F. LOCKETT

From the Department of Physiology, Chelsea Polytechnic, London, S.W.3

(RECEIVED AUGUST 16, 1954)

A sympathomimetic amine, not hitherto described as naturally occurring, was unexpectedly encountered in an experiment in which the adrenaline and noradrenaline, in saline extracts of cat adrenal glands, were being separated chromatographically.

In this experiment a chromatogram, made from an aliquot of extract corresponding to just less than one-third of a cat gland, was oxidized, by spraying with a solution of potassium ferricyanide, to convert any primary or secondary catechol amines to the corresponding adrenochromes. Adrenaline and noradrenaline then appeared as red and pink spots respectively, and were identified by means of their R_F values. There was, however, an additional very pale pink spot (third amine), with an R_F value of approximately 0.7, lying ahead of the adrenaline area from which it was separated by a negative horizontal band of paper. Corresponding bands from another, similar, and adjacent chromatogram were therefore eluted and prepared for biological testing. The eluate from the negative zone separating the adrenaline area from that of the third amine was pharmacologically inert, whereas that from the zone of the third amine was highly active. Moreover, the type of activity encountered in the latter zone differed markedly from that obtained from the adrenaline and noradrenaline areas. The third amine, like adrenaline and unlike noradrenaline, strongly antagonized the action of acetylcholine on the rat uterus. However, when doses of adrenaline and of third amine eluates, which had proved equally active on the rat uterus, were injected intravenously into a cat under chloralose, the adrenaline eluate raised the mean arterial pressure and caused contraction of the nictitating membrane as expected, but the eluate of third amine lowered the blood pressure, caused tachycardia, and was without action on the nictitating membrane.

The results of a further investigation of this third amine are presented here in three sections. The first section is concerned with the great similarity between the third amine and isoprenaline. The second section deals with the presence or absence of the third amine in the adrenal glands of various species. Finally, the properties and actions of the third amine have been contrasted with those of lactyladrenaline and lactylnoradrenaline, because these two compounds are known sometimes to arise as artifacts in extracts of adrenal glands. Lactyladrenaline was first isolated by Kendall (1932) and was studied, together with lactylnoradrenaline, by Crawford (1951). Work by Serlin and Goldenburg (1953) on the instability of synthetic noradrenaline in acid ethanol indicated that these so-called lactyl derivatives may prove to be ethers. Their hypothesis has not been investigated; the terms lactyladrenaline and lactylnoradrenaline are here used to refer to compounds which are believed identical with those previously encountered in extracts of adrenal glands by Crawford.

METHODS

Collection of Adrenal Glands.—Adrenal glands were removed from spinal cats or from cats under anaesthesia (ether, chloroform, chloralose, or pentobarbitone), and from rabbits and guinea-pigs immediately after death from a blow on the head. Cat, rabbit, and guinea-pig glands were at once put into a deep freeze at $-20°$ C. Human and monkey glands were removed under ether anaesthesia, and were quickly carried to the laboratory in dry tubes surrounded with ice in a thermos flask.

Preparation of Solutions for Chromatography.—The procedure described below is that finally adopted and used for glands of all species. Frozen glands were rapidly weighed, cut into small pieces, and ground in ice-cold saline (0.9% NaCl, 2 ml./gland) within 2 hr. of excision from the animal. Coarse debris was removed from the saline extract by centrifuging at 3,000 rev./min. for 3 min. Usually 0.3 ml. M/15 phosphate buffer, *p*H 7.4, and 0.2 ml. 0.9% NaCl were added to each 0.5 ml. aliquot of supernatant fluid; sometimes the buffer was replaced by additional saline. Proteins were then precipitated by the addition of 7 ml. ethanol and 0.25 ml. 4N-HCl.

After centrifuging, 7.5 ml. of supernatant fluid was evaporated to dryness *in vacuo* at a temperature not exceeding 50° C. The residue was extracted with 3 ml. of a mixture of equal parts of acetone and ethanol; 2.5 ml. of this acetone-alcohol extract was evaporated to small volume and used for chromatography.

Chromatography and Elution.—The methods used for chromatography, for the development of chromatograms, for elution, and for the preparation of eluates for biological testing have been described in full detail elsewhere (Vogt, 1952) and need only be summarized here. The concentrated acetone-alcohol extracts were quantitatively transferred to acid-washed Whatman No. 1 filter paper. The area of the paper to be traversed by gland samples destined for use in bioassays had previously been sprayed with a solution of ascorbic acid 50 mg./100 ml. Phenol containing 15% w/v 0.1 N-HCl was used as solvent, and ascending chromatograms were run in an atmosphere of CO_2. Phenol was removed by washing with benzene, and the whole process, including drying, was carried out at room temperature. R_F values were determined from chromatograms developed by spraying with 0.44 g. potassium ferricyanide dissolved in 100 ml. 0.2 M-phosphate buffer, *p*H 7.8.

Adrenaline, noradrenaline, isoprenaline, and lactyladrenaline were used as reference compounds, Chromatograms of not less than two samples from a single gland, and of mixed reference compounds, were made in parallel on a single sheet of paper. The solvent front was allowed to advance 14–16 cm. beyond the line of application of the extracts before the process was interrupted. Chromatograms from one sample of each gland, and of the reference compounds, were used for determination of R_F values; those of other gland samples were eluted and prepared for bioassay as described by Vogt (1952).

Pharmacological Methods.—Adrenaline and noradrenaline were assayed either on the blood pressure and nictitating membrane of a spinal cat, or on the rat uterus and colon as described by Gaddum, Peart, and Vogt (1949). The third amine was compared with isoprenaline in parallel quantitative assays in which at least three—and usually four, and sometimes five—different tissues drawn from two different species were employed. The pharmacological differentiation of the new amine from adrenaline was greatly facilitated by the use of an atypical strain of rats obtained, accidentally, from Taylor (London). The uteri, colons, and ileums of these rats were remarkably insensitive to adrenaline, but were normally sensitive to isoprenaline and to the third amine (Table I). Assays of the third amine on the blood pressure, nictitating membrane, and bronchiolar resistance of cats were carried out under chloralose anaesthesia.

Drugs.—(−)-Adrenaline tartrate (Burroughs Wellcome, Ltd.), (−)-noradrenaline and (±)-isoprenaline (Sterling Winthrop) were obtained commercially. Lactyladrenaline and lactylnoradrenaline were synthesized as described by Crawford (1951).

TABLE I

THE RELATIVE SENSITIVITY OF TISSUES FROM DIFFERENT STRAINS OF RATS TO (−)-ADRENALINE AND (±)-ISOPRENALINE

Rats		Ratio of Equally Effective Doses, (−)-Adrenaline/(±)-Isoprenaline, Expressed as a Range			
Source	No. Examined	Ileum	Colon	Uterus	
Gaddum, Peart and Vogt (1949) ..	—	—	1	1–2	(typical)
Chester Beatty Institute	4	1–50	1–3	1–2	
London School of Pharmacy.. ..	8	1–11	1–3·5	1–3	
Burroughs Wellcome Ltd.	4	1–5	1–2	1–3	
Old assay rats and Taylor (London)..	25	35–65	20–60	12–50 (atypical)	

RESULTS

The Properties of the Third Amine

Pharmacological.—Preliminary experiments had shown that the pharmacological properties of the third amine differed from those of adrenaline and noradrenaline, and resembled those of isoprenaline. Both the third amine and isoprenaline caused a fall

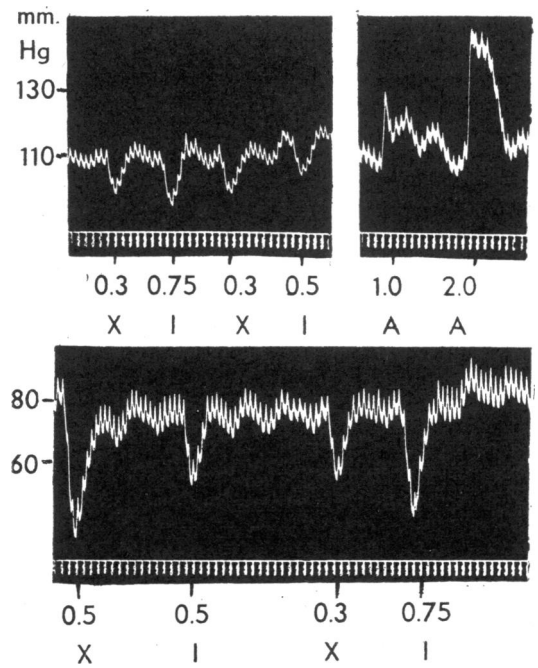

FIG. 1.—Potentiation of the depressor actions of isoprenaline and third amine by tolazoline. Cat, under chloralose. Arterial blood pressure before (upper record) and after (lower record) 7 mg. tolazoline HCl. All drugs injected i.v. A, (−)-adrenaline, in μg.; I, (±)-isoprenaline in μg.; X, an eluate of third amine, from cat adrenal, in ml. Time, 10 sec.

in blood pressure and tachycardia when injected intravenously into spinal cats or cats anaesthetized with chloralose, and their depressor actions were equally potentiated by adrenaline antagonists (Fig. 1). This indicated that both amines have a weak but comparable vasoconstrictor action which is normally masked by their stronger dilator effect, and is abolished by sympatholytic drugs. The effect of third amine on the nictitating membrane varied from cat to cat, but always in parallel with that of isoprenaline. Isoprenaline, 0.5 to 2 μg., was without effect on the nictitating membrane of 10 animals, caused contraction in 7, and relaxation in 3. Cocaine, injected subcutaneously into cats in doses of 5 to 7 mg./kg., affected the responses of the blood pressure and nictitating membranes to the two amines similarly. An initial apparent potentiation of their depressor action was only once observed, and was followed by the typical change seen in each of three other experiments: the depressor actions of these two amines were converted to pressor activities, and their power to cause contraction of the nictitating membrane was potentiated, in parallel (Fig. 4). Further remarkable similarity between the two amines became apparent after the subcutaneous injection of pilocarpine nitrate, 0.5 mg./kg., into cats. Pilocarpine caused great and selective potentiation of the contractions of the nictitating membrane in response to isoprenaline and the third amine (but not in response to adrenaline and noradrenaline), and converted their depressor to pressor activity. Moreover, doses of these two amines, which were equi-effective on the blood pressure and on the nictitating membrane, were equally powerful in the relief of pilocarpine-induced bronchospasm (Fig. 2).

Isoprenaline and the third amine were so alike pharmacologically that they could not be distinguished from one another by means of parallel quantitative assays. Fig. 3 illustrates one such assay, in which an eluate of third amine obtained from cat adrenal gland was compared with isoprenaline. The top records in Fig. 3 show that 1 ml. of this eluate was about equal to 1 μg. (±)-isoprenaline in causing contraction of the nictitating membrane and vasodepression in a cat under chloralose. The centre tracing shows that 1 ml. of the eluate equated with 1 μg. (±)-isoprenaline as an antagonist of acetylcholine on the rat colon. The lowest record demonstrates that 1 ml. of the eluate had more than the activity of 0.7 μg., and less than that of 1.3 μg., (±)-isoprenaline in inhibiting the action of acetylcholine on the rat uterus. Since the ratios of the equiactive doses, (−)-adrenaline/(±)-isoprenaline, were, for these particular tissues, uterus 40–45 and colon 1.0 (see Methods) the activity attributed to the third amine cannot have been due to some adrenaline which had become displaced by an overloading of the paper during chromatography. Fig. 4 shows another comparison, this time between isoprenaline and an eluate of third amine obtained from human adrenal gland. The top records are

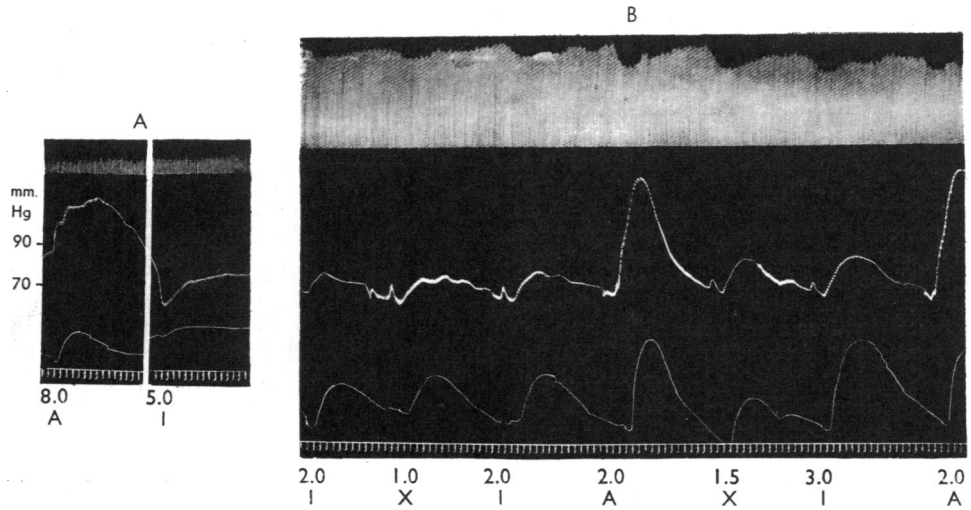

FIG. 2.—Comparison of third amine with isoprenaline, before and after pilocarpine. Cat, under chloralose. Records, from above, are of bronchiolar resistance, arterial blood pressure, and contractions of nictitating membrane —A, before, and B, after, 0.85 mg. pilocarpine nitrate s.c. Other drugs injected i.v. in the doses indicated. A, (−)-adrenaline in μg.; I, (±)-isoprenaline in μg.; X, eluate of third amine, from monkey adrenal, in ml.

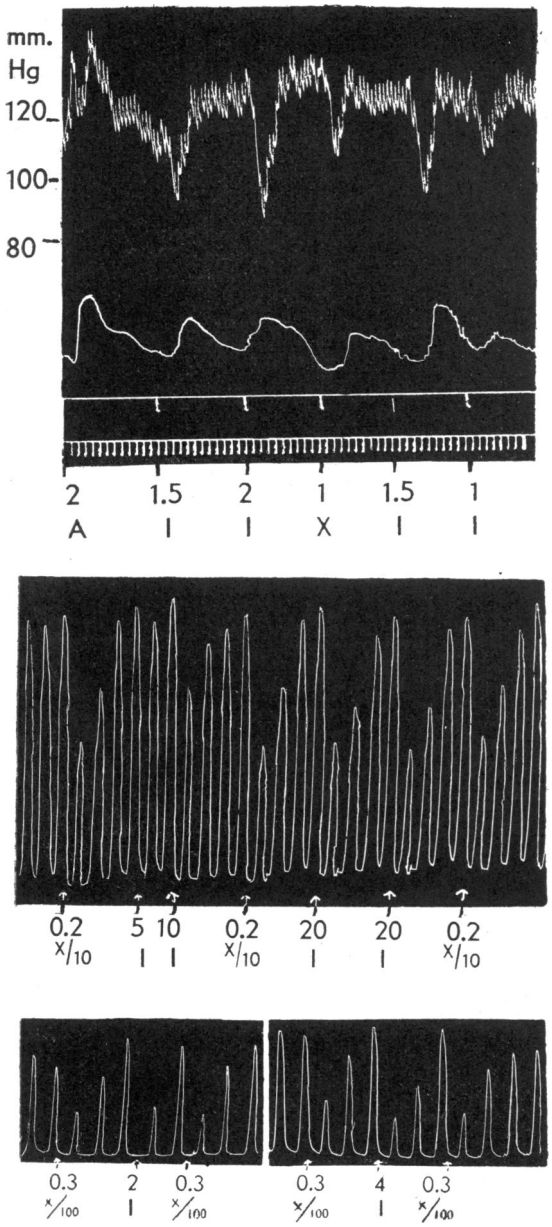

FIG. 3.—Comparison of third amine, from cat adrenal, with isoprenaline, in parallel assays. *Top record*, from cat under chloralose, shows, from above, arterial blood pressure, nictitating membrane, signal, and time in 10 sec. Drugs were injected i.v. Doses of I and of A in μg., and of X in ml. *Middle record*, isolated rat colon responding to 0.1 μg. acetylcholine added at min. intervals, and in contact for 15 sec. At each arrow an inhibitory drug was added 30 sec. before the next addition of acetylcholine. Doses of I in ng., of X in ml. *Bottom record*, rat uterus, contracting every 3 min. in response to 3 μg. acetylcholine acting for 45 sec. Arrows mark additions of inhibitory drugs, 60 sec. before the next addition of acetylcholine. Doses of I in ng., of X in ml. *In all records*, A is (−)-adrenaline, I is (±)-isoprenaline and X is eluate of third amine.

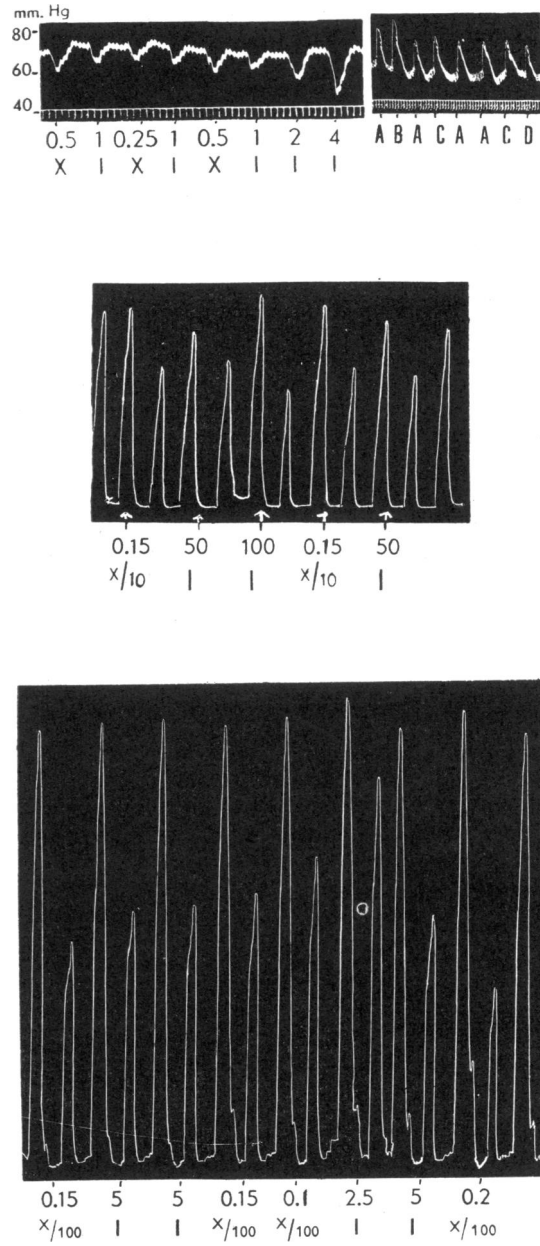

FIG. 4.—Comparison of third amine, from human adrenal, with (±)-isoprenaline, in parallel assays. *Top record*, arterial blood pressure of spinal cat. Drum slowed, and 12 mg. cocaine HCl given s.c., between the 2 parts. Other drugs injected i.v. A, 1.5 μg.; B, 2.5 μg., and D, 1.0 μg., isoprenaline; C, 0.5 ml. eluate of third amine. In *middle record* (rat colon) and *bottom record* (rat uterus) the contractions are in response to a fixed dose of acetylcholine; inhibitory drugs were added as indicated. I, (±)-isoprenaline in μg. in *top record* and in ng. in others; X, eluate of third amine in ml.

of mean arterial pressure, and were taken from a spinal cat weighing 1.9 kg. The first tracing shows that 0.5 ml. of the eluate had more depressor action than 1 μg. and less than that of 2 μg. (±)-isoprenaline. After the subcutaneous injection of 12 mg. cocaine hydrochloride, 0.5 ml. of the eluate had a pressor activity approximately equal to that of 1.5 μg. (±)-isoprenaline. The two lower records show that 0.5 ml. of the eluate equated with 1.7 μg. of (±)-isoprenaline as an antagonist of acetylcholine, both on the rat colon (centre) and on the rat uterus (lowest). The ratios of equally effective doses, (−)-adrenaline/(±)-isoprenaline, were, for these particular rat tissues, uterus 2.0 and colon 56.

The great similarity between isoprenaline and the third amine was not confined to pharmacological activity.

Colour Reactions.—Both third amine and isoprenaline gave green colours with ferric chloride and very strong adrenochrome reactions when treated with oxidizing reagents, and fluorescence was obtained in the ultraviolet for both compounds after treatment with ferricyanide. Adrenochrome formation is typical of catechol amines with primary and secondary amino nitrogen in the side chain, and fluorescence could not occur were the hydroxyl group in the side chain substituted (Lund, 1949).

R_F *Values.*—So great was the similarity between the R_F values obtained for isoprenaline and for the third amine that isoprenaline has been used successfully throughout this work as a chromatographic indicator for the position of the third amine.

Table II summarizes the R_F values which were obtained simultaneously for synthetic compounds and for gland amines. There was no significant difference between the R_F values for natural and synthetic adrenaline and noradrenaline, or between the values obtained for isoprenaline and the third amine.

The Incidence of the Third Amine in the Adrenal Glands of Various Species

The Criteria Adopted for the Identification of the Third Amine.—The existence of the third amine in an extract of adrenal gland was not considered to have been proved until each of three conditions had been satisfied. These criteria were: (i) An R_F value and (ii) a depressor action similar to those of isoprenaline; (iii) an inability to distinguish between the amine and isoprenaline by means of parallel quantitative assays carried out on at least three tissues drawn from two different species. In addition, the tissues selected included either a rat uterus or a rat colon which was abnormally insensitive to adrenaline, but which had normal sensitivity to isoprenaline (see Table I and colon, Fig. 5). The latter precaution was adopted so that no possibility of confusion between adrenaline and the third amine should exist.

The Frequency with which the Third Amine has been Encountered in the Adrenal Glands of Various Species can be read from Table II. This shows R_F values, simultaneously determined, for reference compounds and for gland amines. When a gland chromatogram showed no visible spot for an amine, the R_F value for the corresponding reference compound in that particular experiment was omitted in the compilation of the Table. Since adrenaline was present in every gland chromatogram, the total number of animals in each species that was investigated may be found in brackets following the mean R_F value ± the standard error for gland adrenaline. The number of animals of that species in which third amine was found follows the mean R_F value ± S.E. for third amine in brackets. It was found in the gland extracts from all 4 monkeys, from all 3 human beings, and from three-quarters of the cat population examined. It was occasionally encountered in extracts from rabbit glands, but never in those from guinea-pig glands. Third amine is also

TABLE II

SIMULTANEOUS DETERMINATIONS OF THE R_F VALUES OF SYNTHETIC AMINES AND THOSE OCCURRING IN SALINE EXTRACTS OF ADRENAL GLANDS

(Solvent, phenol containing 15% w/v 0·1N-HCl. For explanation of numerals in parentheses, see text.)

Source	Noradrenaline	Adrenaline	Isoprenaline	Lactyladrenaline
Synthetic	0·210±0·010 (19)	0·486±0·009 (28)	0·684±0·008 (17)	0·801±0·021 (5)
			Third amine	
Cat	0·207±0·007 (12)	0·463±0·019 (12)	0·699±0·026 (9)	0·780±0·051 (2)*†
Monkey	0·203±0·037 (4)	0·459±0·044 (4)	0·672±0·029 (4)	0·820 (1)†
Rabbit	0·210±0·010 (2)	0·504±0·018 (5)	0·675±0·122 (2)	—
Guinea-pig	0·230±0·001 (2)	0·496±0·009 (6)	—	0·780±0·017 (2)**
Man	—	0·490±0·010 (3)	0·707±0·118 (3)	

* Gland stored in deep freeze. † Extract stored overnight in refrigerator after precipitation of proteins with ethanol and acid.

apparently absent from ox adrenal gland. By courtesy of Messrs. Allen and Hanburys Ltd. residues obtained after extraction of the corticoids, and equivalent to a ton of fresh ox adrenal gland, were worked up by Mr. Eastland to an ammoniacal concentrate of a total volume of 2 l. This concentrate yielded 127 g. of crude adrenaline, but neither the crude adrenaline nor the mother liquors contained demonstrable third amine.

The Quantity of Third Amine Present in Extracts of Adrenal Glands.—Only very small amounts of third amine have been encountered. It has been measured as (±)-isoprenaline÷2, and is entered in column 5 of Table III as a percentage of the

TABLE III

THE PERCENTAGE DISTRIBUTION OF SYMPATHOMIMETIC AMINES IN SALINE EXTRACTS OF THE ADRENAL GLANDS OF VARIOUS SPECIES

Third amine measured as (±)-isoprenaline÷2. The values in the Table are the means±their standard errors.

Species, and No. Used	Mg. Amine Extracted from 1 g. Fresh Whole Gland	% Total Extracted Amine		
		(−)-Adrenaline	(−)-Noradrenaline	Third Amine
Cat (12) . .	0·967±0·135	74·37±2·89	23·42±3·23	2·21±0·51
Monkey (4)	1·062±0·350	94·93±0·80	3·72±0·96	1·35±0·43
Man (3) . .	0·123±0·241	86·33±1·34	13·30±0·82	0·53±0·24
Rabbit (5)	0·530±0·111	95·32±0·47	4·44±0·24	0·24±0·42
Guinea-pig (6)* . .	0·351±0·098	93·87±3·32	6·13±3·37	Nil

* Groups of animals.

total amine extracted from the glands of the various species. The second column of this table shows the total weight of amine obtained per gram of fresh gland; the yields were low, indicating that only a part of the medullary amine went into solution during mild extraction with ice-cold saline. The very low values obtained for human gland are probably explained by the fact that two of the three glands were cancerous, and all of them had been cut open and scraped during examination by the surgeons. Glands from other species were always obtained in intact form. The last three columns of Table III record the relative amounts of (−)-adrenaline, (−)-noradrenaline, and third amine found in extracts of the adrenal glands of the different species, expressed as a mean % of the total amine ± the standard error of the mean.

Very small quantities of noradrenaline, which had not been visible on the developed chromatograms, were frequently found on elution of the appropriate areas of parallel chromatograms from human, guinea-pig, and rabbit glands. This noradrenaline was estimated either on the rat colon, or on the blood pressure of a spinal cat, and the identity of the amine was always confirmed by demonstration of a typically weak action as an antagonist of acetylcholine on the rat uterus. Third amine, like isoprenaline, gave a much stronger adrenochrome reaction than noradrenaline, and was therefore more readily visible than the latter amine when present in trace quantities.

The Properties of Lactyladrenaline and Noradrenaline Contrasted with those of the Third Amine

Both lactyladrenaline and lactylnoradrenaline were found in extracts made from a human phaeochromocytoma, which had been cut in pieces and stored in 0.1N-HCl by Crawford in 1951. Crawford obtained R_F values in phenol of 0.62 (lactylnoradrenaline) and 0.75 (lactyladrenaline) both for his synthetic and naturally occurring compounds.

R_F *Values.*—In my experiments the R_F values for synthetic lactyladrenaline and lactylnoradrenaline in phenol have ranged from 0.74–0.86 and 0.55–0.58 respectively, and confirm Crawford's findings. Lactylnoradrenaline was not further examined, because the R_F values obtained in phenol were too low to permit of its identification with the third amine. Lactyladrenaline did, however, require full investigation. The R_F values obtained for synthetic lactyladrenaline have been consistently higher than those for the third amine (Table II), and lactyladrenaline has only been encountered as an artifact either when the glands were stored overnight in the deep freeze, or when extracts from which the proteins had been precipitated with acid alcohol had been stored in the refrigerator. On one occasion both lactyladrenaline and third amine were present in a chromatogram from a stored monkey gland.

Moreover, when an aqueous eluate of chromatographically pure lactyladrenaline (R_F value in phenol 0.79) was re-chromatographed, two spots appeared, one of R_F value 0.80, and another which corresponded in R_F value to the adrenaline reference spot. By contrast, re-chromatography of an eluate of third amine yielded one spot only which had an R_F value identical with that of the original substance.

Colour Reactions.—Whereas both lactyladrenaline and the third amine gave typical adrenochrome reactions, that from the third amine fluoresced in ultraviolet light, but that from lactyladrenaline did not. Moreover, when chromatograms of the two compounds were sprayed first with alkaline hydroxylamine solution and then with acid ferric chloride (Whittaker and Wijesundera, 1952) only with lactyladrenaline

was there any fugitive purple colour amongst the general medley of colours.

Pharmacological Actions.—Lactyladrenaline had only very weak pharmacological actions which resembled those of adrenaline, and which may have been in large part due to partial hydrolysis, of the ester link in aqueous solution (see above). Fig. 5 illustrates the similarity between the effects of adrenaline and of a solution of chromatographically pure lactyladrenaline 500 μg./ml. The top set of records show that 10 μg. (−)-adrenaline and 0.15 ml. of the latter solution caused similar contractions of the nictitating membrane, and had comparable pressor effects, when injected intravenously into an anaesthetized cat. When measured as antagonists of acetylcholine, 10 μg. (−)-adrenaline equated with 0.1 ml. of the lactyladrenaline solution on the rat colon (middle record) and with 0.2 ml. on the rat uterus (bottom record). It was also noticed that rat tissues which were abnormally insensitive to adrenaline were also insensitive to lactyladrenaline (middle record).

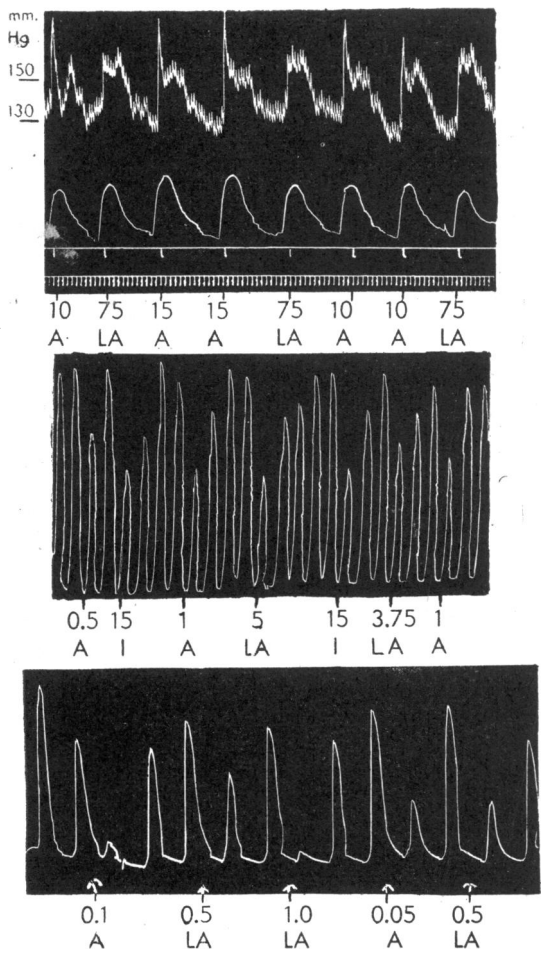

FIG. 5.—Comparison of adrenaline, lactyladrenaline, and isoprenaline. *Top record*, arterial blood pressure and contractions of nictitating membrane (cat under chloralose). *Middle record*, rat colon. *Bottom record*, rat uterus. Contractions of colon and uterus in response to a fixed dose of acetylcholine, and inhibited by the addition of other drugs. A, (−)-adrenaline in μg., LA, lactyladrenaline in μg., I, (±)-isoprenaline in ng.

DISCUSSION

The evidence presented has clearly differentiated the newly discovered third amine both from the medullary hormones (adrenaline and noradrenaline), and from those artifacts (lactyladrenaline and lactylnoradrenaline) which are known to arise in extracts of adrenal glands.

The evidence also indicates that the third amine is not an artifact. Firstly, it was found only in the glands of certain species, although identical mild extraction and separation processes were applied to those of all the species. Secondly, were third amine produced as an artifact, some correlation would be expected either between the total weight of amine extracted from the gland and submitted to chromatography, or between the adrenaline/noradrenaline ratios of the glands, and the incidence of third amine: no such correlation existed. Finally, the very great pharmacological potency of this third amine argues against the artifact hypothesis.

The colour reactions given by the third amine indicate that it is a catechol amine, containing either primary or secondary amino nitrogen and a free hydroxyl group in the side chain. The type of pharmacological action shown by this amine resembles, in general, that of noradrenaline with a single *N*-substituent of chain length equal to or exceeding two carbon atoms (Konzett, 1940b). In particular, the great potency of the third amine as an antagonist of pilocarpine-induced bronchospasm indicates a possible identification with isoprenaline (Konzett, 1940a). This last hypothesis receives great support from the results of a series of parallel quantitative assays which failed to differentiate the third amine from isoprenaline. The acceptance of this identification reveals a hitherto unknown biochemical synthesis, which remains to be investigated.

SUMMARY

1. A hitherto unreported sympathomimetic amine has been detected in saline extracts of adrenal glands from cats, monkeys, and man.

2. This amine was separated chromatographically, and was present only in trace quantity. It has been shown to differ in R_F value, and in pharmacological activity, from adrenaline, noradrenaline, and their lactyl derivatives.

3. The third amine resembles isoprenaline very closely in R_F value, colour reactions, and pharmacological activity. It could not be differentiated from isoprenaline in parallel assays.

The expenses incurred in this work were defrayed by a grant from the Medical Research Council. The equipment was provided by a grant from the Wellcome Trust.

It is a pleasure to thank Miss Ann Hommersham for her skilled assistance.

References

Crawford, T. B. B. (1951). *Biochem. J.*, **48**, 203.

Gaddum, J. H., Peart, W. S., and Vogt, M. (1949). *J. Physiol.*, **108**, 467.

Kendall, E. C. (1932). *J. biol. Chem.*, **97**, v.

Konzett, H. (1940a). *Arch. exp. Path. Pharmak.*, **197**, 27.

—— (1940b). Ibid., **197**, 41.

Lund, A. (1949). *Acta Pharmacol.*, **5**, 121.

Serlin, I., and Goldenburg, M. (1953). *Biochem. J.*, **54**, 483.

Vogt, M. (1952). *Brit. J. Pharmacol.*, **7**, 325.

Whittaker, V. P., and Wijesundera, S. (1952). *Biochem. J.*, **51**, 348.