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IN VITRO EFFECT OF ADRENALINE AND OTHER AMINES ON GLUCOSE METABOLISM IN SHEEP THYROID, HEART, LIVER, KIDNEY AND TESTICULAR SLICES*

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Abstract—Adrenaline, noradrenaline, and serotonine stimulate the oxidation of glucose carbon 1 and glucose carbon 6 by the thyroid tissue of various species *in vitro*. This effect has been related to the activation by these hormones of a DPNH and a TPNH oxidase system. The effect of adrenaline depends on its prior oxidation to adrenochrome. A similar but weaker effect of adrenaline and adrenochrome on glucose carbon 1 oxidation by liver and kidney cortex slices has been observed.

A stimulation of glucose carbon 1 oxidation by substrates of monoamine oxidase has been observed in thyroid and testis slices. While this mechanism accounts for the effect of adrenaline on testis slices it does not account for the effect of this hormone on thyroid slices.

The glycogenolytic effect of adrenaline on liver and heart tissue has been demonstrated by an increased glucose output by liver slices and a decreased glucose uptake by heart slices in the presence of this hormone.

The biological significance of these three *in vitro* actions of adrenaline on glucose catabolism is discussed and it is concluded that only the glycogenolytic effect is physiological.

IT IS generally agreed that the function of the thyroid gland is regulated by the pituitary through the secretion of thyroid stimulating hormone (TSH).[1–4] However, in the 1920s, Cannon *et al.*[5–7] observed that the intravenous injection of adrenaline, and the direct stimulation of the cervical sympathetic system, both elicited a prolonged action current in the thyroid of the cat. They concluded that these two stimulatory agents, enhanced the secretion of the thyroid gland. More recently, Amiragova claimed that the thyroid activity was controlled by the central nervous system, and that this control was mainly mediated by the adrenal medulla.[8, 9]

Adrenaline, noradrenaline and serotonine stimulate the short term release of radioiodine from prelabeled glands.[10–12] The catecholamines alter the thyroid metabolism *in vivo*, in some unknown way.[13] It is thus of interest that adrenaline, noradrenaline and serotonine stimulate *in vitro* the catabolism of glucose in thyroid slices.[14–16] This *in vitro* effect of adrenaline is mainly due to its oxidative derivative: adrenochrome[14, 15] It is the purpose of the present investigation to confirm and extend

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these observations, to compare the effects of adrenaline on the catabolism of glucose by thyroid, heart, kidney cortex, liver, and testicular slices, and to discuss the physiological meaning of these effects.

EXPERIMENTAL

The methods used in this investigation have been previously described.[17] The identification of tissue slices was checked by histology. Incubation of tissue slices was carried out either in a Warburg apparatus or in a Dubnoff metabolic shaker under oxygen or air. The incubation generally lasted 2 hr. Each flask contained 2·6 ml Krebs Ringer phosphate buffer (pH = 7·5), 3·6 mg glucose, 0·8 μc of either [1-^{14}C] or [6-^{14}C] carrier free glucose (Radiochemical Center, Amersham), and 3·2 mg bovine albumin (Armour). The determination of the radioactive yield of $^{14}CO_2$ and the error of the method have been previously reported.[17] The mean effect of any added compound, and the statistical significance of this effect were evaluated by testing the ratios of the observed values in paired control and experimental flasks of individual experiments for deviation from a ratio of unity. The logarithms of the ratios were employed. Student's t values together with mean effects, were calculated from the mean, and standard error of the mean, of the log form of these ratios.[18, 19] When no probability is indicated, the results represent the logarithmic means of at least three agreeing duplicates.

Spectrophotometric studies were made on Beckman model DU spectrophotometer. l-Adrenaline and dichloro*iso*propylnoradrenaline were kindly provided by U.C.B. (Brussels). The other amines were purchased from Calbiochem (Los Angeles). Dihydroergotamine and iproniazid were kindly furnished by Sandoz Cy. and Roche Cy. (Basel) respectively. Adrenochrome, purchased from Calbiochem (Los Angeles), was used for the majority of the experiments. The commercial product, solubilized in methanol was precipitated with an excess of ether at 4°. This partially purified adrenochrome had the same absorbancy spectrum as the commercial product. It was used to confirm the effects of adrenochrome on thyroid and liver tissue.

RESULTS

Adrenaline and glucose metabolism in thyroid slices

l-Adrenaline stimulates the oxidation of glucose carbon 1 and of glucose carbon 6 in sheep thyroid slices (Fig. 1)*. This effect is significant ($P \leqslant 0{\cdot}05$) at a concentration of $1{\cdot}23\ 10^{-5}$ M. The stimulation of glucose carbon 1 oxidation is much stronger than the stimulation of glucose carbon 6 oxidation. Noradrenaline, adrenochrome and serotonine stimulate in the same way the catabolism of glucose by sheep thyroid slices (Table 1). Adrenaline and noradrenaline also enhance glucose carbon 1 oxidation and glucose carbon 6 oxidation by horse, pig and calf thyroid slices. Dog thyroid

* The following abbreviations have been used in this article:
EMKP: Embden–Meyerhof Krebs pathway.
HMP: Hexose monophosphate pathway.
TSH: Thyroid stimulating hormone.
MAO: monoamine oxidase.
TPN$^+$ and TPNH are the oxidized and reduced forms of triphosphopyridine nucleotide.
DPN$^+$ and DPNH are the oxidized and reduced forms of diphosphopyridine nucleotide.
Phenylephrine: l-*m*, hydroxy (methylaminomethyl)-benzyl Alcohol hydrochloride.
Glucose carbon 1 and glucose carbon 6 oxidation are the radioactive yields of $^{14}CO_2$ produced by the tissue slices from (1–^{14}C) glucose and (6–^{14}C) glucose respectively.
When data are given with asterisks *, ** or ***, it means that these data are significant to the level $P < 0{\cdot}05$, $P \leqslant 0{\cdot}01$ or $P \leqslant 0{\cdot}001$ respectively.

slices react poorly to adrenaline.[19] They are not stimulated by adrenaline 10^{-3} M, but react to a concentration of $5 \cdot 10^{-3}$ M of this compound.

Dihydroergotamine $3 \cdot 4\ 10^{-4}$ M does not significantly inhibit the effect of adrenaline upon glucose carbon 1 oxidation by sheep thyroid slices. (Table 2). Dihydroergotamine *per se* has a slight stimulatory effect. Dichloro*iso*propylnoradrenaline $2 \cdot 10^{-3}$ M partially inhibits the stimulating effect of adrenaline 10^{-3} M. This compound by itself

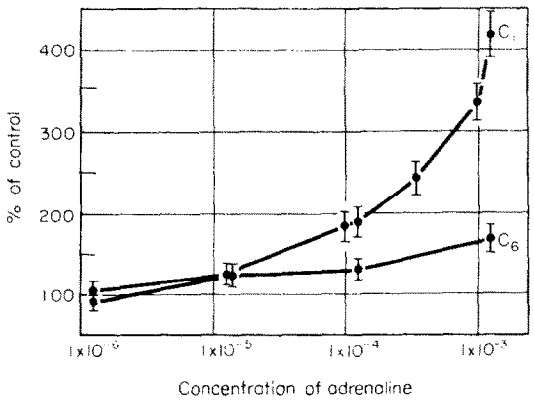

FIG. 1. Stimulation by adrenaline of glucose carbon 1 and glucose carbon 6 oxidation by sheep thyroid slices. The effect of adrenaline is expressed in per cent of the values obtained for the control flasks.

TABLE 1. EFFECT OF AMINES ON GLUCOSE CARBON 1 AND GLUCOSE CARBON 6 OXIDATION BY SHEEP THYROID SLICES. ACTION OF IPRONIAZID ($5 \cdot 10^{-3}$ M) ON THIS EFFECT

Amines	Glucose C6	Glucose C1		N	P
		Without iproniazid	With iproniazid		
Control	100	100	117	15	$\leqslant 0 \cdot 01$
l-Adrenaline 10^{-3} M	155	231	201	9	$\leqslant 0 \cdot 2$
l-Noradrenaline $2 \cdot 10^{-3}$ M	140	265	175	5	—
Adrenochrome $5 \cdot 10^{-4}$ M	160	230	337	3	—
Tyramine $5 \cdot 10^{-3}$ M	—	295	104	7	$\leqslant 0 \cdot 001$
Amphetamine $5 \cdot 10^{-3}$ M	—	170	166	2	—
Serotonine $5 \cdot 10^{-3}$ M	—	168	181	2	—
Serotonine 10^{-3} M	123	152	172	2	—
Phenylethylamine $5 \cdot 10^{-3}$ M	—	251	106	2	—
Hydroxyphenylethylamine $5 \cdot 10^{-3}$ M	—	196	—	3	—
n-Amylamine $5 \cdot 10^{-3}$ M	—	209	99	2	—
p-Hydroxyphenylacetic acid $5 \cdot 10^{-3}$ M	—	110	102	2	—

Results are expressed in per cent of the values obtained for the control flasks.
Results of the experiments with and without iproniazid are directly comparable.
N: number of experiments.

stimulates glucose carbon 1 oxidation at lower concentrations and depresses it at higher concentrations.

In one experiment adrenaline, phenylephrine and noradrenaline, at a concentration of $6 \cdot 2\ 10^{-4}$ M enhanced glucose carbon 1 oxidation to 166, 159 and 130 per cent of the control value respectively.

TABLE 2. EFFECT OF DIHYDROERGOTAMINE (DHE) UPON ADRENALINE STIMULATED THYROID SLICES (GLUCOSE CARBON 1 OXIDATION)

Concentration of adrenaline	Control	Adrenaline	DHE $3{\cdot}4\ 10^{-4}$ M	Adrenaline and DHE	N
l-Adrenaline 10^{-4} M	100	179	147	171	4
l-Adrenaline $3{\cdot}4\ 10^{-4}$ M	100	185	130	157	8
l-Adrenaline 10^{-3} M	100	205	104	170	4

Results are expressed in per cent of the values obtained for the control flasks.

Monoamine oxidase and glucose metabolism in thyroid slices

Tyramine, phenylethylamine, hydroxyphenylethylamine and norleucamine, markedly stimulate the oxidation of glucose carbon 1 by sheep thyroid slices (Table 1). For three of these compounds, the effect was completely inhibited by iproniazid. *p*-Hydroxyphenylacetic acid does not enhance this variable. Iproniazid *per se* slightly stimulates glucose carbon 1 oxidation and markedly increases glucose carbon 6 oxidation by sheep thyroid slices (150 per cent). The stimulation of glucose carbon 1 oxidation by amphetamine and serotonine is not inhibited by iproniazid while the stimulatory effect of adrenochrome is enhanced by this compound. The effect of adrenaline and noradrenaline seems slightly inhibited by iproniazid. However this inhibition is not significant.

Adrenochrome formation in the incubation medium

During the incubation of adrenaline in the presence or absence of thyroid slices, the incubation medium turned pink. No change in colour was observed in anaerobiosis. A spectrophotometric analysis of the medium before incubation shows a maximum of absorbancy at 280 mμ. During incubation the absorbancy increases and shifts to 300 mμ, and a new absorbancy band appears at 485 mμ (Fig. 2). This new spectrum

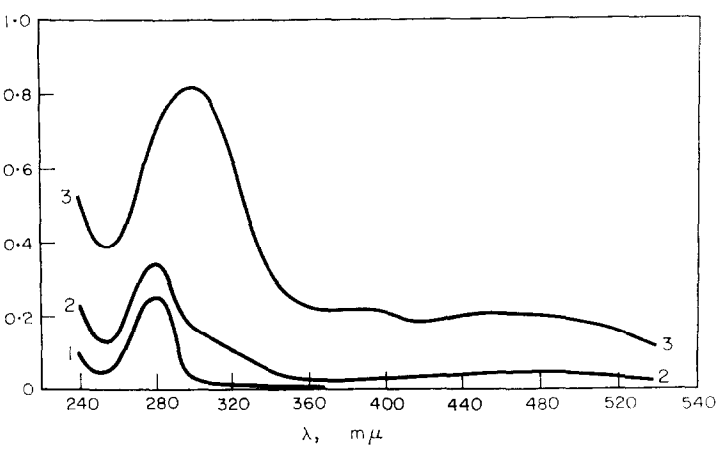

FIG. 2. Absorption spectra of adrenaline during incubation
1. absorption spectrum at the beginning of the incubation (characterized adrenaline).
2. absorption spectrum after 1 hr incubation.
3. absorption spectrum after 2 hr incubation.

characterizes the oxidation of adrenaline to adrenochrome.[20-22] This oxidation is completely inhibited by sodium bisulfite and partially inhibited by ascorbic acid (Table 3). Similar concentrations of sodium bisulfite and ascorbic acid almost completely inhibit the stimulating effect of epinephrine on glucose carbon 1 oxidation. The stimulating effect of adrenochrome is markedly depressed by sodium bisulfite, while ascorbic acid is completely inactive in this regard. The two reducing agents do not inhibit the stimulating effect of noradrenaline and serotonine on glucose carbon 1 oxidation by sheep thyroid slices (Table 4).

TABLE 3. INHIBITION BY SODIUM BISULFITE AND ASCORBIC ACID OF THE OXIDATION OF ADRENALINE TO ADRENOCHROME

Natrium bisulfite		Ascorbic acid	
Concentration	Optical density at 485 mμ	Concentration	Optical density at 485 mμ
$5 \cdot 10^{-3}$ M	0·005	$2 \cdot 8\ 10^{-3}$ M	0·010
10^{-3} M	0·005	$5 \cdot 6\ 10^{-4}$ M	0·025
$5 \cdot 10^{-4}$ M	0·005	$2 \cdot 8\ 10^{-4}$ M	0·070
10^{-4} M	0·175	$5 \cdot 6\ 10^{-5}$ M	0·300
$5 \cdot 10^{-5}$ M	0·290	$2 \cdot 8\ 10^{-5}$ M	0·370

The concentration of adrenaline was 10^{-3} M. (2 hours incubation).

TABLE 4. INHIBITION BY SODIUM BISULFITE AND ASCORBIC ACID OF THE STIMULATING EFFECT OF BIOLOGICALLY ACTIVE AMINES UPON THE GLUCOSE CARBON 1 OXIDATION BY SHEEP THYROID SLICES

Reducing agent	Control	1-Adrenaline 10^{-3} M	Adrenochrome $5 \cdot 10^{-4}$ M	1-Noradrenaline $2 \cdot 10^{-3}$ M	Serotonine 10^{-3} M
Control	100	334	293	179	199
Bisulfite 10^{-3} M	131	190	155	162	167
Bisulfite $5 \cdot 10^{-4}$ M	115	186	232		
Ascorbic acid $5 \cdot 6\ 10^{-4}$ M	132	174	317	165	187

Results are expressed in per cent of the values obtained for the control flasks.

Adrenochrome is a very labile compound.[20, 23, 24] It is degraded during the incubation, as it is evidenced by a progressive change of its U.V. absorption spectrum (Fig. 3). Thyroid slices were preincubated with unlabeled glucose for 2 hr. After that time [1 — ^{14}C] glucose was added to the medium and the incubation was carried out for another 45 min. When adrenaline was added before the preincubation its effect was much higher than when it was added with the labeled glucose. On the contrary the effect of adrenochrome was much depressed by the preincubation (Table 5). Thus, while the preliminary oxidation of adrenaline considerably enhances the effect of this drug, the degradation of adrenochrome markedly reduces its stimulating effect.

In a short term experiment (45 min of incubation) adrenaline and adrenochrome stimulated glucose carbon 1 oxidation to 171 and 253 per cent of the control flasks respectively.

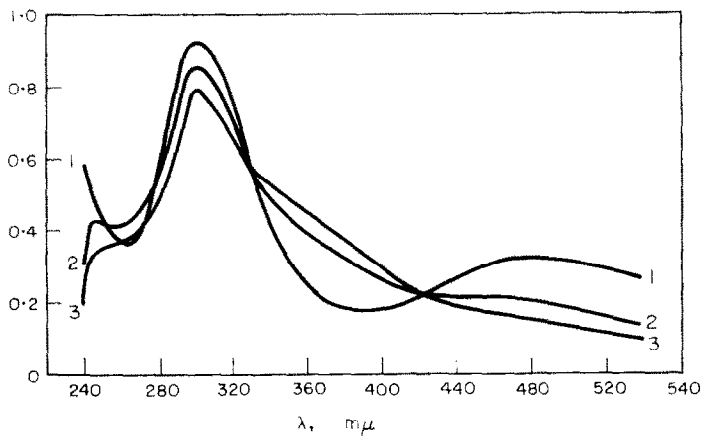

FIG. 3. Degradation of adrenochrome during incubation
1. absorption spectrum of the medium at the beginning of the incubation (characteristic of adrenochrome).
2. absorption spectrum of the medium after 1 hr of incubation.
3. absorption spectrum of the medium after 2½ hr of incubation.

TABLE 5. INFLUENCE OF A PREINCUBATION ON THE STIMULATING EFFECT OF ADRENALINE AND ADRENOCHROME ON GLUCOSE CARBON 1 OXIDATION BY THYROID SLICES

	Compound added		
	Control	l-Adrenaline 10^{-3} M	Adrenochrome $4 \cdot 10^{-4}$ M
Preincubation with the compound	100	1320	188
Preincubation without the compound	100	379	326

Results are expressed in per cent of the values obtained for the control flasks.

Effect of metabolic inhibitors

The stimulating effect of the studied amines on glucose carbon 1 oxidation by sheep thyroid slices has been evaluated in the presence of some specific inhibitory factors. The effect of adrenaline, adrenochrome, noradrenaline and serotonine is not inhibited by fluoroacetate but is moderately inhibited by potassium cyanide. Adrenaline and serotonine do not modify at all the oxidation of glucose carbon 1 in anaerobiosis. Adrenochrome, on the contrary, has a slight, but significant stimulating action (Table 6). During the incubation of thyroid slices with adrenochrome in anaerobiosis, the medium turns yellow indicating a reduction of this compound.[25] This is comparable to the bleaching of methylene blue which occurs in the same conditions.

The metabolic inhibitory factors thus influence the stimulatory effects of adrenochrome, exactly in the same way as they influence the effect of methylene blue or synkavit. The inhibitory pattern of the TSH effect is quite different.[26]

The stimulatory effect of tyramine is not influenced by fluoroacetate but it is inhibited by cyanide. This is consistent with the fact that cyanide directly inhibits monoamine oxidase.[27]

TABLE 6. INFLUENCE OF METABOLIC INHIBITORS ON THE STIMULATING EFFECT OF BIOLOGICALLY ACTIVE AMINES UPON GLUCOSE CARBON 1 OXIDATION BY SHEEP THYROID SLICES

	Control	KCN $5 \cdot 10^{-3}$ M	Fluoroacetate 10^{-2} M	Nitrogen atmosphere
Control	100	38	103	15
l-Adrenaline 10^{-3} M	188***	176***	163	14
l-Noradrenaline 10^{-3} M	122 (2)	93 (2)	108 (2)	—
Serotonine 10^{-3} M	140 (3)	83 (2)	207 (2)	13 (3)
Adrenochrome $5 \cdot 10^{-4}$ M	215***	165**	262	31*
Tyramine $5 \cdot 10^{-3}$ M	265* (4)	75 (4)	252	—
Synkavit $9 \cdot 10^{-5}$ M	620* (4)	373* (4)	642 (2)	22

Results are expressed in per cent of the values obtained for the control flasks.

Glucose carbon 6 oxidation was respectively depressed to 15 per cent, 23 per cent, 0 per cent, of its original value by the addition of cyanide, or fluoroacetate, or by a strict anaerobiosis.

When few experiments were made the number of experiments performed is indicated between parentheses. The results are statistically compared to the corresponding results of the control line when 4 or more experiments were made.

Effect of the amines on glucose catabolism by other sheep tissues

Glucose carbon 1 oxidation is slightly stimulated in liver slices, but more markedly enhanced in kidney cortex slices and testis slices by adrenaline. On the other hand, adrenaline depresses glucose carbon 6 oxidation in liver and both glucose carbon 6 and glucose carbon 1 oxidation in heart slices. Tyramine stimulates glucose carbon 1 oxidation in testis, but depresses both glucose carbon 1 and glucose carbon 6 oxidation in kidney and heart slices. The effect of tyramine on the metabolism of the kidney is reversed by iproniazid (Table 7).

Adrenochrome enhances glucose carbon 1 oxidation in liver, kidney and testis slices, but not in heart slices. The stimulating effect of adrenaline on liver and kidney metabolism is inhibited by bisulfite, while the effect of the hormone on testis is inhibited by iproniazid. The mean ratios C6/C1 of glucose carbon 6 oxidation to glucose carbon 1 oxidation were 0·29, 0·93, 1·04, 0·80, for the liver, kidney cortex, heart and testis respectively.

Adrenaline stimulates the net glucose output by liver slices and decreases the glucose uptake by heart slices. On the contrary adrenochrome decreases the glucose output by liver and does not significantly modify the glucose uptake by heart (Table 8).

TABLE 7. INFLUENCE OF SYMPATHICOMIMETIC AMINES AND OF BISULFITE AND IPRONIAZID ON GLUCOSE CARBON 1 AND GLUCOSE CARBON 6 OXIDATION BY VARIOUS SHEEP TISSUES

		Iproniazid $5 \cdot 10^{-3}$ M	l-Adrenaline 10^{-3} M	l-Adrenaline and Iproniazid	Tyramine $5 \cdot 10^{-}$- M	Tyramine and Iproniazid	Adreno chrome $5 \cdot 10^{-4}$ M	l-Adrenaline 10^{-3} M	l-Adrenaline and bisulfite	Sodium bisulfite 10^{-3} M
Liver	C1	121	126*	118	130	121	155	160	110	78
	C6	100	73*	81	70	80	81			
Kidney	C1	113	142***	127	80	105	140	129	88	110
	C6	102	112	117	63	106	99			
Heart	C1	93	75*	62	64	78	114			
	C6	48	80	64	74	85	56			
Testis	C1	88	170	84	260	101	173	128	135	86
	C6	105	103	78	95	89	54			

Results are expressed in per cent of the values obtained for the control flasks, and statistically compared to these values.
The results of the first 6 columns were not obtained in the same experiments as the results of the last 3 columns.

DISCUSSION

Glucose catabolism proceeds via two pathways at least in thyroid tissue: the Embden–Meyerhof–Krebs pathway (EMKP) and the hexose monophosphate pathway (HMP).[28–30] It has been shown previously that, under the conditions of our experiments, glucose carbon 6 oxidation by thyroid slices reflects the activity of EMKP,

TABLE 8. INFLUENCE OF ADRENALINE AND ADRENOCHROME ON THE NET GLUCOSE RELEASE BY LIVER SLICES AND ON THE GLUCOSE UPTAKE BY HEART SLICES

	Control	1-Adrenaline 10^{-3} M	Adrenochrome $5 \cdot 10^{-4}$ M
Net glucose release by liver	100	119**	69**
Glucose uptake by heart	100	65	110

Results are expressed in per cent of the values obtained for the control flasks.

while glucose carbon 1 oxidation reflects the activity of both the EMKP and the HMP.[17, 30] Adrenaline, noradrenaline and serotonine markedly stimulate glucose carbon 1 oxidation and to a lesser extent glucose carbon 6 oxidation by sheep thyroid slices. A similar effect of adrenaline has been observed in the thyroids of various species. This confirms previously published results.[14–16] The biologically active amines thus stimulate markedly the HMP and to a lesser extent the EMKP in thyroid tissue.[17, 30]

The magnitude of the phenylephrine effect in comparison to the effect of adrenaline and noradrenaline on the catabolism of glucose by thyroid suggested a so called α effect, but the inhibition of this adrenaline effect by dichloro*iso*propylnoradrenaline rather suggested a β effect.[31] The experiments with dihydroergotamine as inhibitor were not conclusive, possibly because of the low dihydroergotamine adrenaline ratio. An effect of adrenaline on a phosphorylase activating mechanism in thyroid[32] is unlikely, since an increased glycogenolysis would tend to dilute the labeled glucose taken up by the slices, to reduce this uptake, and consequently to depress rather than to enhance the radioactive yield of $^{14}CO_2$ from [1 — ^{14}C] and [6 — ^{14}C] glucose. Thus, the known physiological actions of adrenaline did not seem to account for the stimulation by this compound of glucose catabolism in thyroid.

As MAO activity has been demonstrated in thyroid tissue,[33, 34] amines are probably deaminated by this enzyme and H_2O_2 is produced by the reaction. Such a reaction could explain the stimulation by amines of the HMP in thyroid as in pituitary tissue.[35] In fact, substrates of MAO, phenylethylamine, tyramine, norleucamine, hydroxyphenylethylamine all markedly stimulate the HMP in thyroid; *p*-hydroxyphenylacetic acid, on the contrary, has no such effect. The stimulating effect of these amines is completely inhibited by iproniazid, a potent inhibitor of MAO.[36] However, iproniazid does not inhibit the stimulating action of adrenaline, noradrenaline, serotonine or adrenochrome on the HMP in thyroid. It is not as substrates of the thyroid MAO that the latter compounds stimulate the thyroid metabolism.

The effect of adrenaline on thyroid mainly depends on its previous oxidation. This is evidenced by the following facts:

(1) adrenaline is spontaneously oxidized to adrenochrome during the incubation. Adrenochrome *per se* stimulates the metabolism of thyroid slices as does adrenaline. It is more active in short term experiments even though it is rapidly destroyed.[21]

(2) Ascorbic acid and sodium bisulfite which prevent the oxidation of adrenaline,[32] markedly inhibit the stimulating effect of this hormone. Anaerobiosis which also prevents the oxidation of adrenaline completely suppresses any stimulating action of the hormone on the thyroid HMP.

(3) The stimulating effect of adrenochrome is inhibited by bisulfite which combines with adrenochrome,[38] while it is not inhibited by ascorbic acid which only reduces this compound.[23, 39, 10]

(4) Preincubation with adrenaline, during which oxidation to adrenochrome takes place, considerably enhances the stimulating effect of the hormone. On the contrary, preincubation with adrenochrome, during which this compound is degraded, markedly reduces its stimulating effect.

It is thus mainly after its oxidation to adrenochrome that adrenaline stimulates the HMP in thyroid. The hexose monophosphate pathway in thyroid is rate limited by the TPN^+ supply in this tissue.[17, 41–43] Therefore adrenochrome, noradrenaline and serotonine should in some way increase this supply. As the effect of these compounds is not inhibited by fluoroacetate and only partially inhibited by cyanide it is probably not caused by an increased synthesis of TPN from DPN and ATP. Indeed, the effect of TSH on the thyroid HMP, which is due to an increased synthesis of TPN[11] is nearly abolished by these metabolic inhibitors.[26]

Adrenochrome is an hydrogen carrier.[23, 34] It is thus likely that adrenochrome increases the TPN^+ supply in thyroid by catalyzing the oxidation of TPNH. Indeed, like other electron acceptors, as methylene blue, synkavit and diiodotyrosine[26, 13] adrenochrome is reduced in anaerobiosis while TPN^+ is provided in the cell, as it is evidenced by an increased HMP. That adrenochrome does not act merely as an hydrogen acceptor but as an electron carrier, is shown by its very low stimulating effect in anaerobiosis. Adrenochrome does not by itself catalyze the oxidation of TPNH[22] as it does for ascorbic acid. It is thus concluded that this drug acts as an autooxidizable electron carrier for a TPNH oxidase system. A similar DPNH oxidase system activated by adrenochrome and relatively insensitive to cyanide has been previously evidenced in liver.[20] The fact that adrenochrome also stimulated the EMKP in thyroid suggests that this compound also stimulates a DPNH oxidase system in this tissue. While this work was in progress, Pastan *et al.* arrived at the same conclusions, and they were able to demonstrate by spectrophotometric methods that adrenaline and adrenochrome catalytically stimulate the oxidation of TPNH and DPNH by a thyroidal mitochondrial-microsomal preparation.[11]

Noradrenaline and serotonine stimulate the glucose catabolism in thyroid in the same way as adrenaline. Whether or not these compounds have to be oxidized to be active is not known.

A drenaline stimulates glycogenolysis in several tissues.[32] In these tissues, the uptake of glucose being depressed,[15, 46] adrenaline should decrease the oxidation of exogenous glucose. Indeed in heart tissue, this hormone depresses both the uptake of exogenous glucose and the oxidation of its carbon 1 and carbon 6. Adrenochrome has no such effect on heart slices.

Adrenaline stimulates glycogenolysis in sheep liver slices,[46] as it is evidenced by an increased glucose release. However adrenochrome strongly stimulates glucose carbon 1 oxidation by this tissue. Thus while the glycogenolytic effect of adrenaline on liver slices would tend to decrease the oxidation of the carbon 1 and carbon 6 of exogenous glucose, the oxidative derivative of the hormone would enhance the former variable. Depending on the extent of adrenaline oxidation this hormone will or will not stimulate glucose carbon 1 oxidation by liver slices. This explains why some authors observed such a stimulation[14] while others did not.[47] When adrenaline enhances glucose carbon 1 oxidation by liver slices, this effect is of course inhibited by bisulfite. Glucose carbon 1 oxidation by kidney cortex slices is stimulated by adrenochrome. As adrenaline does not stimulate the formation of cyclic AMP in this tissue,[32] it could be expected that the hormone would stimulate glucose carbon 1 oxidation without depressing glucose carbon 6 oxidation by this tissue. The inhibition of this adrenaline effect by bisulfite suggests that it is caused by the oxidative derivative of the hormone. The oxidative deamination of tyramine by the MAO in the kidney[37] depresses both glucose carbon 1 and glucose carbon 6 oxidation.

Adrenaline markedly stimulates glucose carbon 1 but not glucose carbon 6 oxidation by sheep testicular slices. Barondes *et al.* did not observe such effects in rat testicular tissue.[47] The effect of adrenaline is mimicked by tyramine and adrenochrome. However as the adrenaline effect is completely inhibited by iproniazid but not by bisulfite it is concluded that it is as a substrate of the testicular MAO that this hormone stimulates the glucose catabolism in this tissue.

In this study adrenaline has been shown to influence the *in vitro* pattern of glucose catabolism by tissue slices in three different ways:

(a) as a substrate for monoamine oxidase,

(b) as an activator of a DPNH and TPNH oxidase system,

(c) as an activator of glycogenolysis.

The stimulation of glucose carbon 1 oxidation through an oxidative deamination of adrenaline has been observed in anterior pituitary[35] and in testicular tissue. This *in vitro* effect requires concentrations of hormones (10^{-5} M–10^{-4} M) which are much higher than the concentrations necessary to stimulate other *in vitro* systems (10^{-8} M–10^{-6} M)[31, 48] and than the physiological plasma concentrations of this hormone (10^{-9} M).[49] It is neither specific of adrenaline, since it can be obtained with the other substrates of monoamine oxidase, neither organ specific, since it can be observed for several tissues. It is dependent on the degradation of adrenaline while other *in vivo* effects of this hormone are potentiated when this degradation is inhibited.[50, 51] It is therefore very unlikely that this first *in vitro* effect of adrenaline might have a physiological meaning.

The stimulation of glucose carbon 1 oxidation by adrenaline, through the activation of a TPNH oxidase system has been observed in thyroid and our data suggest that a similar phenomenon may occur in liver and kidney tissue. This *in vitro* effect is not organ specific. It requires higher concentrations than other *in vitro* effects of the hormone. It is caused by adrenochrome, which is no longer believed to be an *in vivo* metabolite of adrenaline.[52] Therefore this *in vitro* effect of adrenaline is not believed to have a physiological meaning.

The effects of noradrenaline and serotonine on thyroid slices also require too high concentrations of hormone to be accepted as possible *in vivo* effects. The hypothesis

that higher concentrations of noradrenaline might be secreted on the spot can be excluded since there are no secreto-motor thyroid nerve fibres.[53]

The glycogenolytic effect of adrenaline is obtained for low concentrations of hormone;[46, 48, 49] it is inhibited *in vitro* by known *in vivo* antagonists of adrenaline;[48] it corresponds to well known *in vivo* effects of the hormone.[32] Alterations in the structure of the molecule modify this effect in the same way as they modify the other physiological effects of this hormone.[32, 48] It is thus believed that the *in vitro* glycogenolytic action of adrenaline has a physiological meaning,[32, 48] contrarily to the two other *in vitro* effects observed in this study.

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