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SOME PHARMACOLOGICAL PROPERTIES OF URIDINE NUCLEOTIDES

BY

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Uridine di-, tri- and monophosphates (UDP, UTP and UMP) contracted the goldfish intestine preparation in that order of decreasing potency. Adenosine triphosphate (ATP) sensitized the gut to UTP and UDP but not to UMP. The fluoro-derivatives of UMP and UTP behaved like the unsubstituted nucleotides on the goldfish intestine but the main effect of 6-azaUDP and large amounts of uracil and uridine was to cause a relaxation. Structure-action relationships are discussed on the basis of these findings. UDPglucose and UDPacetylglucosamine each contracted the goldfish intestine but they were 500-times less active than UDP. Other smooth muscle preparations (tortoise jejunum, rat uterus, guinea-pig ileum and the fowl rectal caecum) contracted to UTP and UDP, but large amounts were needed. The cardiovascular effects in rats of UMP, UDP and UTP were complex and mediated mainly through an action on the peripheral blood vessels. In rats treated with phenoxybenzamine, UMP raised the blood pressure while UDP and UTP first lowered then raised the blood pressure. The fall in blood pressure was not abolished by pronethalol or atropine. The uridine phosphates affected the rat isolated heart only under hypoxic conditions. UTP and UDP dilated the blood vessels of the rabbit ear and UTP was six-times more effective than ATP. UTP and UDP were equiactive in increasing the force of beat of the frog isolated heart. UMP also had an effect if large amounts were given.

Uridine nucleotides together constitute 10 to 15% of the total nucleotide content of liver and brain of mammals and appear to exist in a highly labile form (Hurlbert, Schmitz, Brumm & Potter, 1954 ; Lolley, Balfour & Samson, 1961). Within a few seconds *post mortem*, uridine triphosphate (UTP) and uridine diphosphate (UDP) lose inorganic phosphate with the eventual formation of uridylic acid (uridine monophosphate, UMP ; Mandel & Harth, 1961) and there is evidence that UMP can be further degraded to form uridine and uracil (Smith, 1962).

UTP catalyses biochemical reactions with its high-energy phosphate radical and in addition UTP can form adenosine triphosphate (ATP) by transphosphorylation (Berg & Joklik, 1953). UDP is deeply implicated in the synthesis of glycogen (Leloir & Cardini, 1957), glucuronides and probably polysaccharides (Storey & Dutton, 1955). Recently it was found that very small quantities of UDP and UTP caused a contraction of the goldfish intestine (Gaddum & Smith, 1963). This paper describes some of the pharmacological properties of these important nucleotides.

METHODS

Isolated smooth muscle preparations

The intestine from a goldfish (*Carassius auratus*) was used in a microbath, as described by Gaddum & Szerb (1961), and in the presence of the following blocking agents: hyoscine (0.01 μg/ml.), methysergide (0.25 μg/ml.), dichloroisoprenaline (5 μg/ml.) and ATP (10 μg/ml.), unless stated otherwise.

The jejunum of a tortoise (*Testudo graeca*) was used in a 5 ml. organ-bath at a temperature of 25° C, as described by Toh & Mohuiddin (1958).

A rat uterus strip was used in a heated microbath (Gaddum, unpublished) at a constant temperature between 30 and 35° C in de Jalon solution without aeration. Stilboestrol dipropionate (0.1 mg/kg of body weight) was injected intramuscularly into virgin rats 12 hr before removal of the uterus.

The fowl rectal caecum was used as described by Cleugh, Gaddum, Holton & Leach (1961) in the presence of ephedrine (5 μg/ml.), atropine (0.1 μg/ml.) and methysergide (0.05 μg/ml.).

The guinea-pig ileum was used in Tyrode solution at 37° C in a 5 ml. organ-bath in the presence of atropine (0.1 μg/ml.).

Isolated heart preparations

The rat isolated heart was perfused through the coronary vessels with oxygenated Ringer solution at 30° C. Solutions to be injected were heated to 30° C beforehand and care was taken to eliminate any bubbles of air appearing in the perfusion fluid. Contractions were recorded isometrically.

The frog isolated heart was prepared by the method of Bülbring (1930) and used at room temperature. The perfusion fluid contained (g/l.): NaCl 6.5, KCl 0.14, NaH_2PO_4 0.01, $CaCl_2$ 0.12, glucose 2, and $NaHCO_3$ 0.2. Sometimes the concentration of potassium chloride in this solution was increased to 0.42 g/l. Contractions were recorded isometrically.

The rabbit ear preparation

Rabbits were anaesthetized by the injection of 6 ml./kg of urethane solution (25% w/v) into an ear vein. The opposite ear was used for the experiment. The great auricular nerve to that ear was cut and about 30 min later drugs were injected into a cannulated lingual artery. The effects of these drugs on the light transmitted through the ear were recorded photoelectrically by a system essentially similar to that reported by Holton & Holton (1952). On some occasions phenoxybenzamine (5 mg/kg of body weight) was injected intramuscularly 12 hr before the experiment began.

Rat blood pressure measurements

Rats were anaesthetized by intraperitoneal injection of urethane (0.4 ml./100 g of body weight, using a 25% w/v solution). Blood pressure was recorded from a cannulated carotid artery by a Condon manometer. In some experiments the float on the mercury was attached to a lever with a mirror. The movement of a light was reflected from the mirror on to a photocell, the electrical output of which was amplified and recorded on a fast moving drum. Injections were made into a jugular vein or the left carotid artery.

Drugs

The following derivatives of uracil were used:

Drug	Abbreviation	Source
Uracil	—	Roche Products
Uridine	—	Schwarz Bioresearch
Uridine 5′-monophosphate	UMP	Schwarz Bioresearch
Uridine 2′,3′-cyclic phosphate	—	Schwarz Bioresearch

Drug	Abbreviation	Source
5-Fluorouridine 5′-monophosphate	FluoroUMP	National Cancer Inst.
Uridine 5′-diphosphate	UDP	Schwarz Bioresearch
6-Azauridine diphosphate	AzaUDP	National Cancer Inst.
Uridine 5′-diphosphoglucose	UDPglucose	Sigma Chemical Co.
Uridine 5′-diphospho-*N*-acetylglucosamine	UDPacetylglucosamine	Sigma Chemical Co.
Uridine 5′-triphosphate	UTP	Schwarz Bioresearch
5-Fluorouridine 5′-triphosphate	FluoroUTP	National Cancer Inst.

FluoroUMP, fluoroUTP and azaUDP were from the Cancer Chemotherapy National Service Centre, National Cancer Institute, Bethesda 14, Md., U.S.A., and were made available through the generosity of Dr R. E. Handschumacher of the University of Yale. The weights of these compounds refer to the salts.

Other drugs used included: methysergide (Sandoz), dichloroisoprenaline hydrochloride (I.C.I.), phenoxybenzamine hydrochloride (Smith, Kline & French Laboratories) and ATP (C. F. Boehringer & Soehne).

RESULTS

The effects of uridine nucleotides on the goldfish intestine

Small amounts of UDP and UTP contract the goldfish intestine preparation (Gaddum & Smith, 1963). Table 1 shows the concentrations of uridine and other nucleotides needed to cause small contractions of the goldfish intestine (about

TABLE 1

CONCENTRATIONS OF URACIL, URIDINE AND URIDINE NUCLEOTIDES NEEDED TO CAUSE SMALL MOTOR EFFECTS ON THE GOLDFISH INTESTINE

Responses were contractions, except for those where the concentration is marked by an asterisk, which were biphasic, a contraction followed by relaxation

Compound	Salt	Concentration (μg/ml.)
Uridine 5′-triphosphate	Lithium	0·02
5-Fluorouridine 5′-triphosphate		0·02
Uridine 5′-diphosphate	Lithium	0·004
Uridine 5′-diphosphoglucose	Sodium	2
Uridine 5′-diphospho-*N*-acetylglucosamine	Sodium	2
6-Azauridine diphosphate		0·1*
Uridine 5′-monophosphate	Sodium	10
Uridine 2′,3′-cyclic phosphate	Barium	10
5-Fluorouridine 5′-monophosphate		10
Uridine		1,000*
Uracil		500*

6% of the maximal response). UDP was the most potent of the substances tested but, when combined with a molecule of sugar to form UDPglucose or UDPacetylglucosamine, the complex became approximately 500-times less active. Solutions of UDPglucose and UDPacetylglucosamine (100 μg/ml.) were adjusted to *p*H 4, and half of each solution was heated in boiling water for 10 min, the other half being left at room temperature. All solutions were neutralized and tested on the intestine. The results are shown in Fig. 1. At room temperature and *p*H 4 the potencies of the solutions were unchanged. Heating these acid solutions in a boiling water-bath increased the potency by 2.5- to 5-fold; the complex was partly hydrolysed to UDP by this treatment.

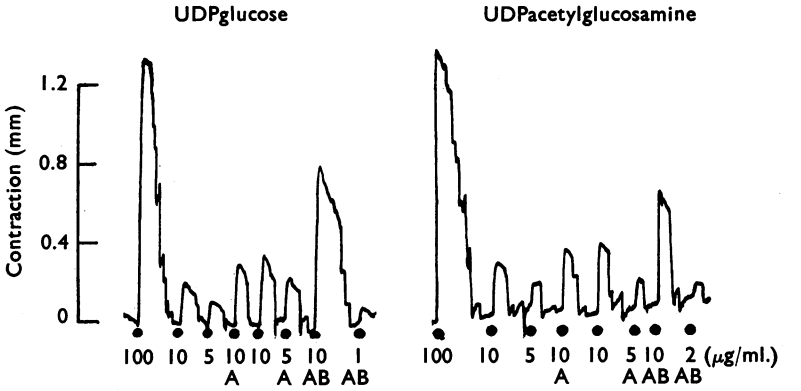

Fig. 1. Contractions of a goldfish intestine preparation in response to uridine diphosphate sugars (concentrations shown in μg/ml.) on, before and after acid treatment. A : solutions kept at *p*H 4 for 10 min at room temperature ; AB : solutions boiled for 10 min at *p*H 4.

Fig. 2 shows the effects of three substituted uridine phosphates on the goldfish intestine. AzaUDP (100 ng/ml.) caused a long lasting relaxation of the intestine. The baseline was adjusted mechanically and threshold concentrations of UMP, UDP and UTP were applied to the gut, followed by a second dose of azaUDP. Relaxation again took place but the effects of UMP, UDP and UTP, given shortly afterwards, were unchanged. At this point azaUDP was added to the bathing fluid

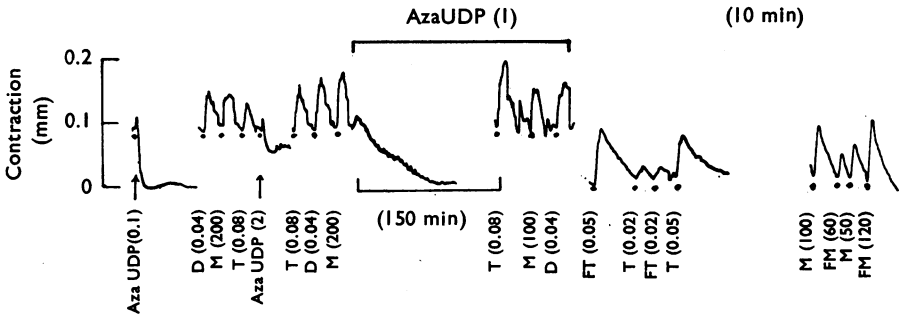

Fig. 2. The actions of substituted uridine nucleotides on a goldfish intestine preparation. M, D and T = UMP, UDP and UTP respectively ; azaUDP = 6-azauridine diphosphate ; and FM and FT = 5-fluorouridine 5′-monophosphate and 5-fluorouridine 5′-triphosphate. The concentrations of nucleotides are given as μg/ml.

in a concentration of 1 μg/ml., twenty-five-times the concentration of UDP which would contract the goldfish intestine. The gut relaxed further but the effects of UMP, UDP and UTP, measured 150 min later, were substantially the same. AzaUDP and UDP appear to produce their effects by different mechanisms. The fluoro-derivatives of UMP and UTP contracted the goldfish intestine (Fig. 2) in the same concentrations as the unsubstituted nucleotides, the triphosphates being 500-times more potent than the monophosphates. Repeated doses of the fluoro-nucleotides did not block the effect of UMP or UTP.

Uracil and uridine were 50- and 100-times less potent than the monophosphate and their main effect was a relaxation. In this respect they resembled the adenine nucleotides.

The action of ATP on the goldfish intestine

The immediate effect of ATP (1 μg/ml.) on the goldfish intestine was a contraction followed by relaxation. If ATP is present in a concentration of 10 μg/ml., added ATP has no effect unless it exceeds the background concentration (Gaddum & Szerb, 1961). ATP in the bathing fluid also partially blocks the effect of added AMP ; it was not known if it altered the response of the intestine to uridine nucleotides. Fig. 3 shows responses of a goldfish intestine to small concentrations of

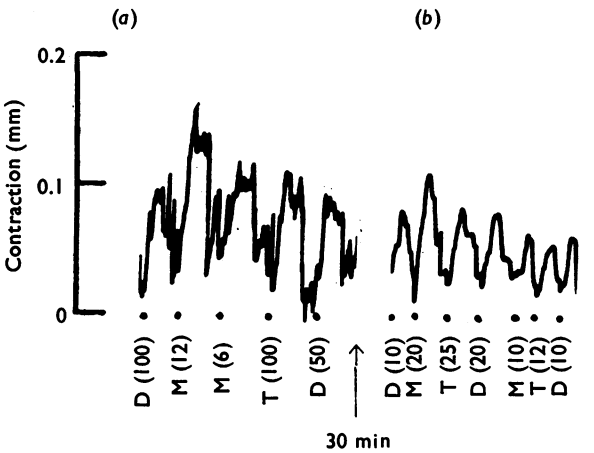

Fig. 3. Responses of a goldfish intestine preparation to uridine phosphates added (*a*) before and (*b*) 30 min after the addition of ATP (10 μg/ml.) to the bathing fluid. M : UMP concentrations given (μg/ml.) ; D and T : UDP and UTP concentrations respectively (ng/ml.).

UMP, UDP and UTP. In the absence of ATP (*a*), the intestine showed spontaneous activity. This was greatly reduced by ATP (10 μg/ml.) added to the bathing fluid and left in contact with the intestine for 30 min (*b*). Besides improving the stability of the tissue, ATP affected the relative potencies of UMP, UDP and UTP as measured by the concentrations of these compounds needed to produce small contractions. The results of three experiments are shown in Table 2. After 30 min contact with ATP the goldfish intestine was more sensitive to UTP and UDP but the threshold dose for UMP remained unchanged. This result might also represent a partial antagonism of UMP by ATP since the sensitivity of the intestine to added drugs sometimes increases after a period of rest. It is clear however that the goldfish intestine reacts in a different way to the di- and triphosphates compared with the monophosphate and that ATP helps to expose this difference.

The log dose/effect curves for UMP and UDP, measured on the goldfish intestine, are plotted in Fig. 4. The lower parts of the curves are very similar but at high concentrations UMP produces a larger contraction than do corresponding doses of

TABLE 2

CONCENTRATIONS OF URIDINE NUCLEOTIDES NEEDED TO PRODUCE SMALL CONTRACTIONS OF THE GOLDFISH INTESTINE

The nucleotides were each tested on three pieces of intestine in the absence and then in the presence of ATP (10 μg/ml.). Dichloroisoprenaline (5 μg/ml.) methysergide (0·25 μg/ml.) and hyoscine (0·01 μg/ml.) were present in the bath fluid throughout every experiment

Nucleotide	Concentration (ng/ml.) required	
	Without ATP	With ATP
Uridine 5′-monophosphate	5,000	10,000
	20,000	20,000
	10,000	5,000
Uridine 5′-diphosphate	50	10
	20	0·1
	5	0·2
Uridine 5′-triphosphate	100	12
	50	5
	20	0·2

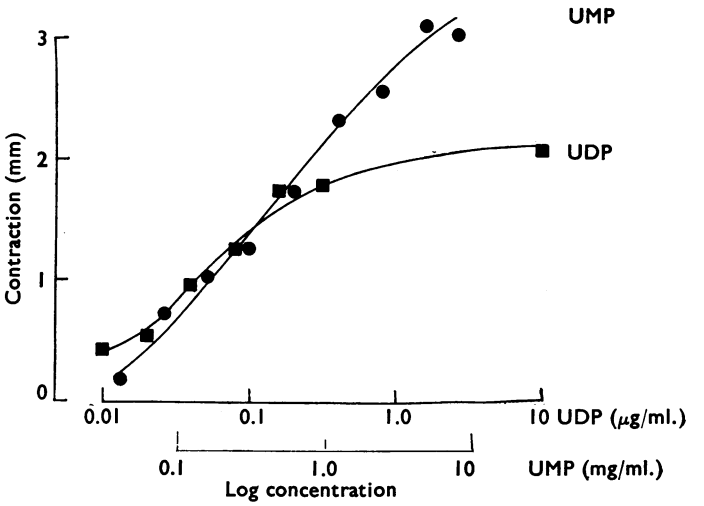

Fig. 4. Log dose effect curves for UMP and UDP measured on a goldfish intestine.

UDP. Using high doses of these two phosphates a further difference is seen in the shape of the response, the contraction to UMP being larger than that to UDP but not as well maintained. UTP behaves very like UDP in this respect.

The effect of uridine phosphates on other smooth muscle preparations

The tortoise jejunum contracts in the presence of small amounts of ATP and other nucleotide triphosphates (Toh, 1963). This preparation and other smooth muscles responded to the uridine phosphates (Table 3), the triphosphate being the most active, but the concentrations needed to produce a small contraction were very much higher than those needed to contract the goldfish intestine.

The cardiovascular actions of the uridine phosphates

It has been widely reported that UTP dilates coronary blood vessels and causes hypotension when injected into anaesthetized animals (Wolf & Berne, 1956 ; Lévy

& Michel-Ber, 1959; Coirault, Lévy, Michel-Ber, Masbernard, Desclos de la Fonchais & Mazingant, 1960b). This effect on blood pressure was confirmed in rats anaesthetized with urethane. Intravenous injections of UTP caused a definite fall in blood pressure, but after treatment with phenoxybenzamine (1 mg/100 g

TABLE 3

APPROXIMATE CONCENTRATIONS (μg/ml.) OF URIDINE NUCLEOTIDE NEEDED TO CAUSE SMALL CONTRACTIONS OF VARIOUS SMOOTH MUSCLE PREPARATIONS

Preparation	Nucleotide		
	UMP	UDP	UTP
Tortoise jejunum	>40	2	0·8
Rat uterus	>10,000	1,000	10
Fowl rectal caecum	400	50	40
Guinea-pig ileum	>260	>100	40

body weight) 12 hr beforehand, UTP raised the blood pressure. UDP and UMP were as active as UTP in raising the blood pressure of the rats treated with phenoxybenzamine. However, as with the goldfish intestine, the dose/effect curve for UMP differed from those for UDP and UTP (Fig. 5). The site of action of these compounds was determined by a method described by Euler & Gaddum (1931). The

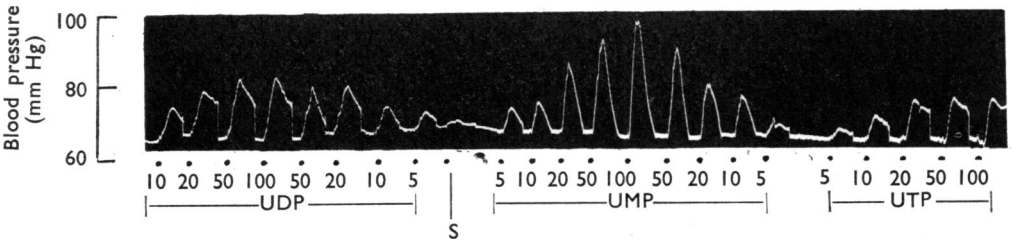

Fig. 5. Record of the arterial blood pressure of a rat (250 g) anaesthetized with urethane (0·4 ml. of a 25% w/v solution/100 g, intraperitoneally), which had been injected intramuscularly with phenoxybenzamine (1 mg/100 g) 12 hr previously. All injections were made into the cannulated left jugular vein, and doses are in μg. ; S = 0·2 ml. of 0·9% saline.

blood pressure was recorded from the right carotid artery on a fast moving chart and the effect of injections into the left jugular vein was compared with that of retrograde injections into the left carotid artery; control injections of 0.9% saline were also used. All injections were of the same volume and were made as quickly as possible. Fig. 6 shows the results for UMP and UDP. UMP had a pure pressor effect which occurred more quickly and was larger when the injection was given into the left carotid artery. This result is evidence that the pressor effect was due to a peripheral action and not due to an action on the heart. In the same way and for the same reasons the initial depressor effect of the other two nucleotides also appeared to be peripheral in origin. UDP and UTP both caused a rise in blood pressure following the initial fall and the magnitude of each phase was influenced by the resting level of the blood pressure; if this was high then the dominant effect was a fall in blood pressure, and *vice versa*.

These pressor effects could not have been mediated through released sympathomimetic amines since the sympathetic α-receptors were blocked by phenoxybenz-

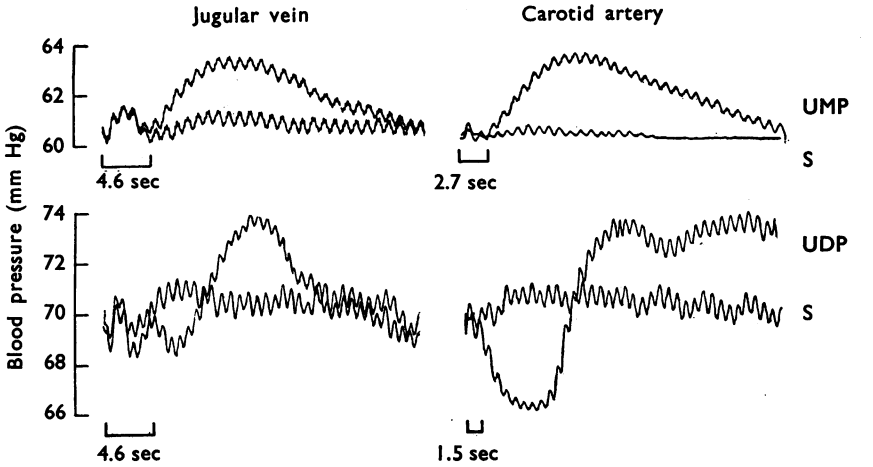

Fig. 6. Superimposed pairs of records of the arterial blood pressure of a rat (300 g) anaesthetized with urethane, comparing responses to injections (at zero times) into the right external jugular vein (left-hand records) and the left common carotid artery (right-hand records). The rat had previously been treated with phenoxybenzamine. Injections were 0·05 ml. in volume and were made as quickly as possible. Intra-arterial injections were made towards the aorta, and intravenous injections towards the heart. S = 0·05 ml. of 0·9% saline. UMP was given in doses of 25 μg, and UDP of 50 μg, by each route.

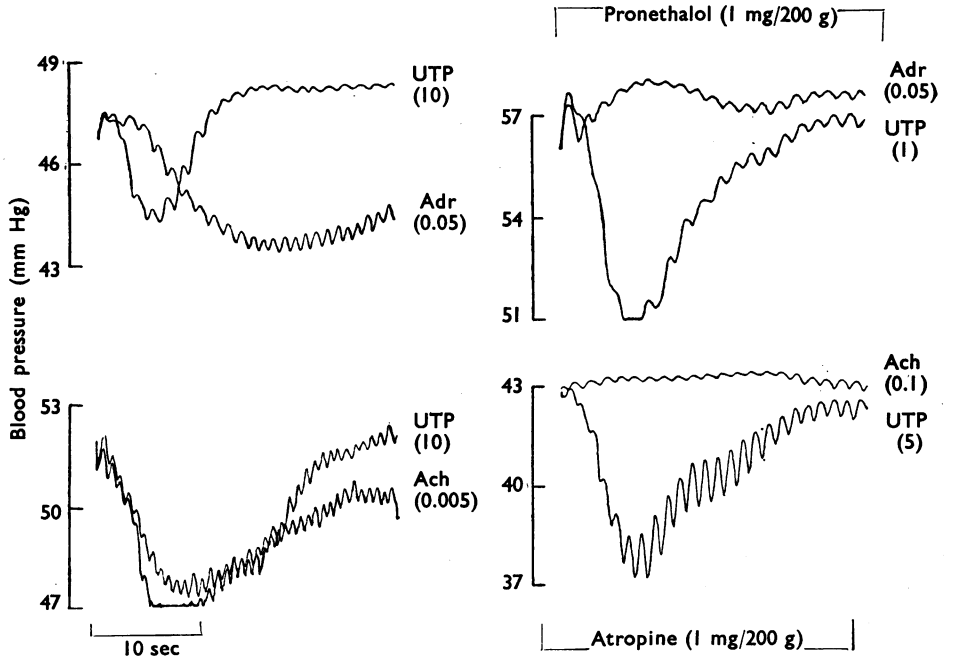

Fig. 7. Record of the arterial blood pressure of a rat (220 g) anaesthetized with urethane and previously treated with phenoxybenzamine. Adr = adrenaline ; Ach = acetylcholine. All injections were made retrogradely into the left carotid artery. The rat had received pronethalol (1 mg/200 g) before the right-hand upper record, and atropine (1 mg/200 g) before the right-hand lower record. Doses of other drugs are in μg.

amine but it was possible that the hypotensive phase of response, seen with UDP and UTP only, might have been due to released adrenaline acting on β-receptors or to released acetylcholine. Adrenaline (50 ng) injected retrogradely into the left carotid artery of a rat treated with phenoxybenzamine caused a fall in blood pressure which was more prolonged than the effect of 10 μg of UTP (Fig. 7). There was no pressor response to adrenaline, which showed that the phenoxybenzamine was fully effective. At this point pronethalol (1 mg/200 g) was injected intravenously and the rat left for 10 min. During this time the resting blood pressure rose to a new steady level. Injected adrenaline now had no hypotensive effect, pronethalol having blocked the β-receptors, but UTP in one-tenth of its former dose produced a fall of 6 mm Hg in blood pressure. Acetylcholine (5 ng), injected into a carotid artery of a second rat treated with phenoxybenzamine, produced a fall in blood pressure similar in size to that due to 10 μg of UTP (Fig. 7). Atropine (1 mg/200 g) abolished the effect of 100 ng of acetylcholine injected 10 min later, while 5 μg of UTP still produced hypotension. It was therefore concluded that these nucleotides produced their constrictor and dilator effects probably on peripheral blood vessels and in a way different from the actions of acetylcholine and adrenaline.

The effect of uridine nucleotides on the rabbit ear

Holton & Holton (1954) tested UMP for its vasodilator effect on the rabbit ear and found it to be inactive. They did not test the di- and triphosphates. Fig. 8 shows the results of injecting the three uridine phosphates into the lingual artery of a rabbit anaesthetized with urethane. In this experiment phenoxybenzamine (0.5 mg/100 g) had been injected intramuscularly 12 hr previously but in other rabbits this was not done and the results were similar. In all experiments the great auricular nerve was cut approximately 30 min before the injection of nucleotides. Dilatation of the blood vessels was recorded as a change in the intensity of filtered light falling on a photocell after passing through the ear. It was confirmed that UMP was inactive, 400 μg producing no dilatation of the ear vessels. UDP was effective in a dose of 200 μg, making it about fifty-times less active than ATP. UTP was a more potent vasodilator than ATP on this preparation, 200 ng producing a small effect. UTP and ATP were compared with each other in a series of 2+2 assays in several rabbits. The mean result of eight assays was that UTP was 7 ± 1.2 (standard error) times more active than ATP. On a molar basis UTP was six-times more active than ATP as a vasodilator.

The action of uridine phosphates on the rat isolated heart

UMP, UDP and UTP had no effect on the rate or amplitude of contraction of the rat heart perfused by the method of Langendorff, provided the coronary circulation was adequately perfused. Neither could a change in coronary flow, measured by counting outflow in drops/min, be detected under these conditions. In an experiment where the coronary flow was artificially controlled by altering the hydrostatic pressure, there was initially a coronary flow of 0.4 ml./min, sufficient to maintain a heart rate of 80 to 84 beats/min for an indefinite time without a reduction in the force of contraction. Injected UMP was inactive under these conditions. When the coronary flow was reduced to 0.34 ml./min the force of contraction fell but could

be partially restored by the injection of 1 mg of UMP (10 to 100 μg of UDP or UTP had similar effects). Further reduction of the coronary flow to 0.2 ml./min slowed the heart rate to 40 beats/min and now UMP appeared to increase the rate of failure. When the coronary flow was artificially restored to 0.34 ml./min the force of contraction improved and 0.1 and 1 mg of UMP again caused a further

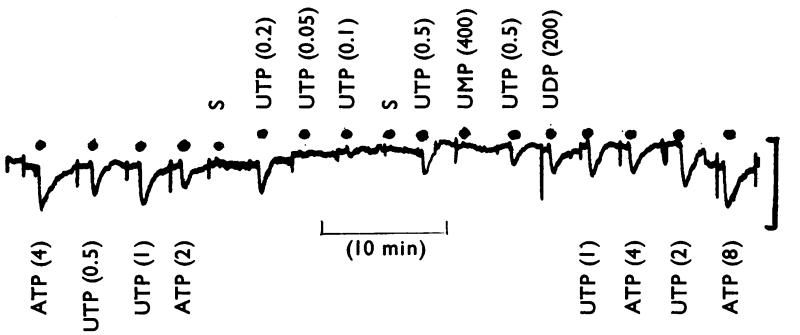

Fig. 8. Record of the transmission of light through the ear of a rabbit (2·4 kg) anaesthetized with urethane (16 ml. of a 25% w/v solution injected intravenously) and injected wth phenoxybenzamine (5 mg/kg) 12 hr previously. ATP was given as the sodium salt, UMP, UDP and UTP as the lithium salts ; S = 0·1 ml. of 0·9% saline. The numbers indicate doses (μg) of the nucleotides. Time calibration, 10 min. The ordinate calibration indicates a 5% drop in light transmitted with respect to the base line.

improvement in the force of contraction. The changes in rate of heart beat after these large doses of nucleotide were small and variable. Effects of the uridine nucleotides on the rat isolated heart are slight, are only seen with large doses and are not likely to be important unless the coronary flow is abnormal.

The action of uridine nucleotides on the frog isolated heart

Uridine nucleotides were tested on a frog heart prepared according to the method of Bülbring (1930) and perfused with a balanced salt solution containing either 73 mg/l. of potassium (Fig. 9, *a*) or 292 mg/l. (Fig. 9, *b*). Each of the three nucleotides increased the force of contraction but UMP was 100-times less active than the di- or triphosphate. In a solution containing the higher concentration of potassium the frog heart ceased to beat and then UDP and UTP restored the

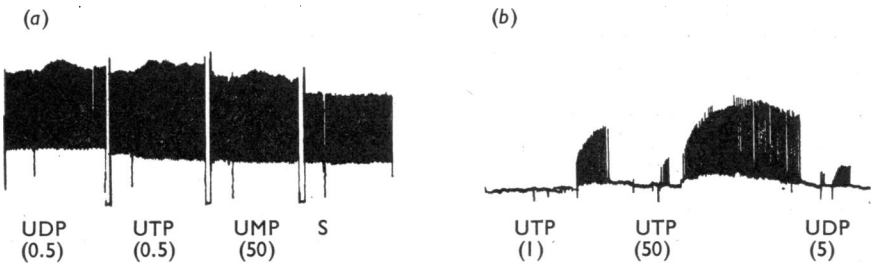

Fig. 9. Isometric contractions of a perfused frog heart. Using (*a*) 1·9 and (*b*) 7·5 mM-potassium in the perfusion fluid. Doses of nucleotides are in μg ; S = 0·05 ml. of perfusate.

beat for various periods of time (Fig. 9, *b*). The higher concentration of potassium made the action of UDP and UTP more noticeable but higher doses (5 to 50 μg) often had to be used to show this effect.

DISCUSSION

Uridine has very little effect on the goldfish intestine unless large amounts are added when the gut gives a small contraction and then relaxes. The addition of two phosphate groups to uridine produces a compound which is 250,000-times more active and which causes a contraction of the intestine with no obvious relaxation phase. The substitution of an azo group at position 6 in UDP produces an inactive molecule which behaves like uridine when tested on the goldfish intestine. The phosphate end of the UDP molecule is necessary to produce a muscular contraction but only when joined to the uridine base which, at first sight, might appear unimportant since it produces no appreciable effect by itself. The potent vasodilator effect of ATP seems to depend on a similar relationship between, in this instance, the 6-amino group of adenine and phosphate groups (Wolf & Berne, 1956). It is these groups which bind to myosin (Blum, 1955) and G-actin (Strohman & Samorodin, 1962) and it may be that uridine phosphates act by such means. Certainly UTP is as effective as ATP in causing contraction and relaxation of glycerinated skeletal muscle (Ranney, 1954). Binding might equally well occur at the cell membrane possibly producing an effect by changes in sodium extrusion across the membrane, since this appears to be controlled by ATPase activity (Skou, 1957). The situation is complex and the information insufficient to come to any more detailed conclusions about structure-action relationships.

The dose/effect curve for UMP was different from those for UDP and UTP when measured on the goldfish intestine and UMP was 1,000-times less active in producing a small contraction. Either UMP acts by a different mechanism or the effects of UDP and UTP are more complicated than they appear (compare their action on the rat blood pressure).

The sugar complexes of UDP contracted the goldfish intestine but an 0.2% contamination with UDP would account for their activity and it would be unwise to attribute any effect to the phosphate sugars on this evidence since they are easily hydrolysed. Heating these compounds at *p*H 4 in a boiling water-bath for 10 min caused some hydrolysis and it now seems very probable that the increase in potency, seen after acid boiling of liver homogenates by Gaddum & Smith (1963), was due to hydrolysis of these uridine sugars.

When ATP was added to the solution bathing the goldfish intestine spontaneous contractions were abolished, the tissue became more reliable for quantitative work and more sensitive to UTP and UDP. It is not known how these effects were produced but it is possible that ATP acted at the cell membrane because it only passes through cell membranes with difficulty (Hasselbach & Weber, 1955). This sensitization is probably not specific for nucleotides; ATP makes a frog rectus muscle more sensitive to applied acetylcholine (Feldberg & Hebb, 1947).

UTP is a potent vasodilator (Wolf & Berne, 1956; Lévy & Michel-Ber, 1959; Coirault *et al.*, 1960a). Results reported here show that UDP also has vasodilator properties and that both UDP and UTP can raise blood pressure under certain conditions. The effect on blood pressure of any injection of UDP or UTP represents the sum of these antagonistic effects and the net result depends on whether the animal has been injected with phenoxybenzamine and on the route of administration. These nucleotides produce a change in blood pressure primarily by affecting peripheral blood vessels and not the heart. However the hypoxic heart will improve when UTP is present (Cascio, 1962) and a similar result is seen with all three phosphates when the perfusion flow of the rat isolated heart is reduced to cause either hypoxia or starvation. Part of this improvement can be explained by the definite increase in metabolic rate which UTP produces (Wolfe & Berne, 1956). UMP has a pressor effect in the rat without any preliminary vasodilatation and again the action is on the peripheral blood vessels. The vasodilator effect of UTP is probably direct on the blood vessel wall; pronethalol and atropine do not affect this hypotension.

UTP was six-times more potent than ATP as a vasodilator in the rabbit ear. This is the first nucleotide reported to have a greater vasodilator effect than ATP. Holton & Holton (1954) postulated that ATP might be the chemical transmitter responsible for the vasodilatation seen on antidromic nervous stimulation and Holton (1959) identified ATP liberated from sensory nerves following stimulation. However ATP can be made from UTP by transphosphorylation (Berg & Joklik, 1953) and the question arises whether this process could occur in the rabbit ear. An analysis of spinal roots for UTP would perhaps be worth while. A further complication in the assay of ATP has recently arisen due to the identification of transphosphorylases in the firefly lantern (Balfour & Samson, 1959). It was previously thought that this assay method was specific for ATP.

Isolated frog hearts are very sensitive to uridine nucleotides and show an increase in the force of beat to 0.5 μg of UDP or UTP, and this confirms the work of Lévy & Michel-Ber (1959). UMP produces a similar effect to UDP and UTP but 100-times more material is needed. Uridine has also been shown to increase the tension of isolated strips of the frog ventricle (Cook, Greene & Lorber, 1958).

There seems no doubt that uridine and its phosphates have important physiological functions. Uridine will counteract acute left ventricular failure in the dog isolated heart (Buckley, Tsuboi & Zeig, 1959) and will restore abnormal carbohydrate metabolism in the perfused cat brain (Geiger & Yamasaki, 1956), while UTP has been used with some success in the treatment of myasthenia, poliomyelitis and other diseases characterized by muscular weakness (Coirault *et al.*, 1960b).

REFERENCES

BALFOUR, W. M. & SAMSON, F. E. (1959). Transphosphorylases in the firefly lantern. *Arch. Biochem.*, **84**, 140–142.

BERG, P. & JOKLIK, W. K. (1953). Transphosphorylation between nucleoside polyphosphates. *Nature (Lond.)*, **172**, 1008–1009.

BLUM, J. J. (1955). The enzymatic interaction between myosin and nucleotides. *Arch. Biochem.*, **55**, 486–511.

BUCKLEY, N. M., TSUBOI, K. K. & ZEIG, N. J. (1959). Effects of nucleosides on acute ventricular failure in the isolated dog heart. *Circulat. Res.*, **7**, 847–857.

BÜLBRING, E. (1930). Die Wirkung einiger neuerer Herzmittel am durchströmten Froschherz. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **152**, 257–272.

CASCIO, G. (1962). Influenza dell'uridin-5-trifosfato sulla resistenza del miocardio alla sottrazione totale o parziale di O_2. *Boll. Soc. ital. Biol. Sper.*, **38**, 1244–1246.

CLEUGH, J., GADDUM, J. H., HOLTON, P. & LEACH, E. (1961). Assay of substance P on the fowl rectal caecum. *Brit. J. Pharmacol.*, **17**, 144–158.

COIRAULT, R., LÉVY, J., MICHEL-BER, E., MASBERNARD, A., DESCLOS DE LA FONCHAIS, S. & MAZINGANT, F. (1960a). L'acide uridine-5-triphosphorique (U.T.P.) en therapeutique. 1-Traitement des atrophies musculaires neurogènes. *Presse Méd.*, **68**, 1127–1129.

COIRAULT, R., LÉVY, J., MICHEL-BER, E., MASBERNARD, A., DESCLOS DE LA FONCHAIS, S. & MAZINGANT, F. (1960b). L'acide uridine-5-triphosphosrique (U.T.P.) en therapeutique. 2-Traitement des myopathies, myotonies, myasthénies et des états de fatigue. *Presse Méd.*, **68**, 1169–1171.

COOK, M. H., GREENE, E. A. & LORBER, V. (1958). Effect of purine and pyrimidine ribosides on an isolated frog ventricle preparation. *Circulat. Res.*, **6**, 735–739.

EULER, U. S. VON, & GADDUM, J. H. (1931). An unidentified depressor substance in certain tissue extracts. *J. Physiol. (Lond.)*, **72**, 74–87.

FELDBERG, W. & HEBB, C. (1947). The effects of magnesium ions and of creatinine phosphate on the synthesis of acetylcholine. *J. Physiol. (Lond.)*, **106**, 8–17.

GADDUM, J. H. & SMITH, M. W. (1963). A pharmacologically active substance in mammalian tissue extracts. *Proc. roy. soc. B*, **157**, 492–506.

GADDUM, J. H. & SZERB, J. C. (1961). Assay of substance P on goldfish intestine in a microbath. *Brit. J. Pharmacol.*, **17**, 451–463.

GEIGER, A. & YAMASAKI, S. (1956). Cytidine and uridine requirement of the brain. *J. Neurochem.*, **1**, 93–100.

HASSELBACH, W. & WEBER, A. (1955). Models for the study of the contraction of muscle and of cell protoplasm. *Pharmacol. Rev.*, **7**, 97–117.

HOLTON, P. (1959). The liberation of ATP on antidromic stimulation of sensory nerves. *J. Physiol. (Lond.)*, **145**, 494–504.

HOLTON, F. A. & HOLTON, P. (1952). The vasodilator activity of spinal roots. *J. Physiol. (Lond.)*, **118**, 310–327.

HOLTON, F. A. & HOLTON, P. (1954). The capillary dilator substances in dry powders of spinal roots ; a possible role of adenosine triphosphate in chemical transmission from nerve endings. *J. Physiol. (Lond.)*, **126**, 124–140.

HURLBERT, R. B., SCHMITZ, H., BRUMM, A. F. & POTTER, V. R. (1954). Nucleotide metabolism. II. Chromatographic separation of acid-soluble nucleotides. *J. biol. Chem.*, **209**, 23–39.

LELOIR, L. F. & CARDINI, C. E. (1957). Biosynthesis of glycogen from uridine diphosphate glucose. *J. Amer. chem. Soc.*, **79**, 6340–6341.

LÉVY, J. & MICHEL-BER, E. (1959). Propriétés pharmacologiques de l'uridine triphosphate. *C.R. Acad. Sci., (Paris)*, **248**, 2416–2417.

LOLLEY, R. N., BALFOUR, W. M. & SAMSON, F. E. (1961). The high-energy phosphates in developing brain. *J. Neurochem.*, **7**, 289–297.

MANDEL, P. & HARTH, S. (1961). Free nucleotides of the brain in various mammals. *J. Neurochem.*, **8**, 116–125.

RANNEY, R. E. (1954). Dissociative and contractile reactions of actomyosin with uridine triphosphate. *Amer. J. Physiol.*, **178**, 517–520.

SKOU, J. C. (1957). The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. biophys. Acta (Amst.)*, **23**, 394–401.

SMITH, K. C. (1962). Catabolism of derivatives of uracil and cytosine by rat tissues. *J. Neurochem.*, **9**, 277–280.

STOREY, I. D. E. & DUTTON, G. J. (1955). Uridine compounds in glucuronic acid metabolism. *Biochem. J.*, **59**, 279–288.

STROHMAN, R. C. & SAMORODIN, A. J. (1962). The requirements for adenosine triphosphate binding to globular actin. *J. biol. Chem.*, **237**, 363–370.

TOH, C. C. (1963). Biologically active substances in brain extracts. *J. Physiol. (Lond.)*, **165**, 47–61.

TOH, C. C. & MOHUIDDIN, A. (1958). Vasoactive substance in the nasal mucosa. *Brit. J. Pharmacol.*, **13**, 113–117.

WOLF, M. M. & BERNE, R. M. (1956). Coronary vasodilator properties of purine and pyrimidine derivatives. *Circulat. Res.*, **4**, 343–348.