ANTAGONISM TO THE ACTIONS OF HYDRALLAZINE, RESERPINE, POTASSIUM CYANIDE, SODIUM AZIDE AND ANOXIA ON ARTERIAL SMOOTH MUSCLE

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Received November 18, 1957

Many intermediates of carbohydrate, fat and protein metabolism give protection against the hydrallazine depression of acetylcholine, (−)-adrenaline, (−)-noradrenaline, histamine and 5-hydroxytryptamine-induced contractions of spirally cut strips of horse carotid arteries. Strips made anoxic behave in a manner similar to hydrallazine-treated strips. Inhibition of drug-induced contractions by potassium cyanide, iodoacetate and azide was of a different character from that caused by hydrallazine and anoxia. Few intermediates gave significant protection. Hydrallazine probably exerts its effect by a non-specific depression of metabolism rather than upon specific receptors. Reserpine depression of drug-induced contractions in artery strips was so persistent that experiments using intermediary metabolites could not be made.

HYDRALLAZINE and reserpine antagonise contractions induced by acetylcholine, (−)-adrenaline, (−)-noradrenaline, histamine, 5-hydroxytryptamine and potassium chloride on spirally cut strips of horse, cat and rabbit arteries[1,2]. Antagonism to barium chloride has been observed only on strips of horse carotid arteries. These observations demonstrated that both reserpine and hydrallazine lacked specificity of action[2–6]. Reserpine appears to have a strong affinity for arterial smooth muscle and it is very difficult to reverse its effects. These observations and those of Gillis and Lewis[3–6] have indicated that we may be dealing with an effect upon cellular mechanisms connected with the production and utilisation of energy necessary for drug induced contractions rather than with effects upon specific receptors[2]. We have attempted to antagonise the depressant effects of hydrallazine and reserpine upon drug induced contractions and to imitate their effects by using potassium cyanide, sodium azide and sodium iodoacetate, or by rendering the tissue anoxic.

MATERIALS AND METHODS

The bath fluid was oxygenated Tyrode's solution at 36° of the following composition in g./l., NaCl 8·0, KCl 0·198, $CaCl_2$ 0·2, NaH_2PO_4 0·05, $NaHCO_3$ 1·0, glucose 1·0.

Drugs used were acetylcholine chloride (ACh), (−)-adrenaline hydrochloride (Ad), (−)-noradrenaline bitartrate (NA), 5-hydroxytryptamine creatinine sulphate (5-HT), histamine acid phosphate (Hm), potassium cyanide (KCN), sodium azide, sodium iodoacetate, sodium monofluoroacetate, glutathione, *p*-chloromercuribenzoate, 1-hydrazinophthalazine hydrochloride (hydrallazine), reserpine (as a buffered solution in

ascorbic acid-sodium ascorbate) and sodium thiocyanate. Substances used to antagonise hydrallazine and reserpine inhibition of drug-induced contractions are detailed in Tables I to III. Except for maleic acid (pH 1·8) and (±)-leucine (pH 1·0) they were added to the bath as neutral

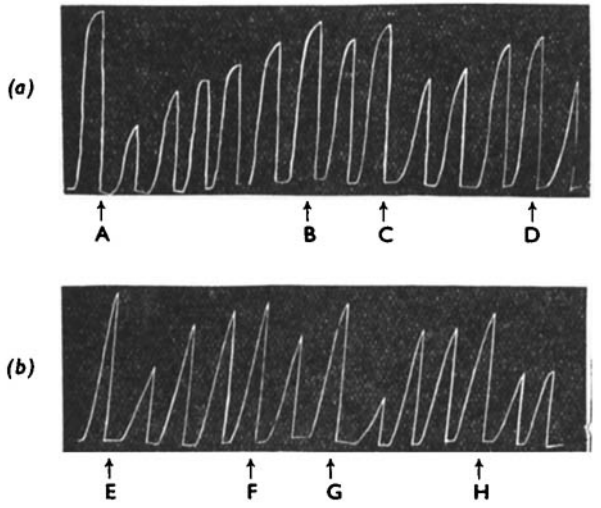

FIG. 1. The effects of pyruvate, succinate, *cis*-aconitate, fumarate, citrate and oxaloacetate on hydrallazine inhibition of ACh-induced contractions of strips of horse carotid artery. (*a*) All contractions due to 0·13 μg. ACh. At A, 0·13 mg. hydrallazine. At B, 1 mg. pyruvate and 0·13 mg. hydrallazine. At C, 1 mg. succinate and 0·13 mg. hydrallazine. At D, 1 mg. fumarate and 0·13 mg. hydrallazine. (*b*) All contractions due to 0·02 μg. ACh. At E, 0·066 mg. hydrallazine. At F, 1 mg. oxaloacetate and 0·066 mg. hydrallazine. At G, 1 mg. *cis*-aconitate and 0·066 mg. hydrallazine. At H, 1 mg. citrate and 0·066 mg. hydrallazine.

solutions of the pure substance to avoid pH effects. All drug concentrations refer to the final bath concentrations per ml. With compounds shown in Tables I to III this was one mg.

Spirally cut strips of common carotid artery, from horses just killed were set up in 10 to 75 ml. organ baths. The experimental procedure has already been described[2]. In testing for antagonism to the actions of reserpine and hydrallazine, the antagonists (Table I) were added to the bath 10 minutes before the addition of reserpine or hydrallazine and remained in contact with the tissue for 30 minutes.

Changes of pH were minimised by using neutral solutions as far as possible. Since the contents of the bath were a complex mixture of salts and drugs and the possibility of chemical inactivation was present, some experiments were made in which hydrallazine did not come into contact with the added compound. The chemical was allowed to remain in the bath for 10 minutes and was then washed out. Hydrallazine was

now added and the experiment continued. Release of Hm from the tissue might also result in an apparent antagonism to hydrallazine. We therefore repeated some experiments using Tyrode's solution containing 100 μg./l. of mepyramine maleate.

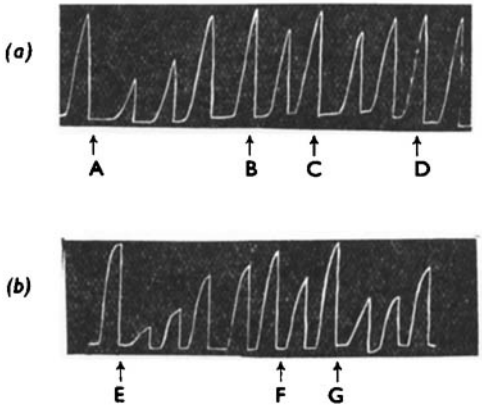

FIG. 2. The effects of 3-phosphoglycerate, (±)-alanine, butyrate, pyruvate and succinate on hydrallazine inhibition of Ad and NA induced contractions of strips of horse carotid artery. (*a*) All contractions due to 0·2 μg. Ad. At A, 0·26 mg. hydrallazine. At B, 1 mg. 3-phosphoglycerate and 0·26 mg. hydrallazine. At C, 1 mg. (±)-alanine and 0·26 mg. hydrallazine. At D, 1 mg. butyrate and 0·26 mg. hydrallazine. (*b*) All contractions due to 0·13 μg. NA. At E, 26 μg. hydrallazine. At F, 1 mg. pyruvate and 26 μg. hydrallazine. At G, 1 mg. succinate and 26 μg. hydrallazine.

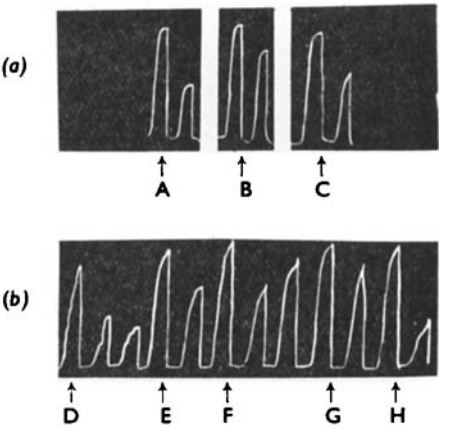

FIG. 3. The effects of pyruvate, succinate, (±)-alanine, glutamate, α-ketoglutarate and fumarate upon KCN inhibition of ACh-induced contractions of strips of horse carotid artery. (*a*) All contractions due to 0·2 μg. ACh. At A, 0·1 mg. KCN. At B, 1 mg. pyruvate and 0·1 mg. KCN. At C, 1 mg. succinate and 0·1 mg. KCN. (*b*) All contractions due to 0·1 μg. ACh. At D, 0·15 KCN. At E, 1 mg. (±)-alanine and 0·15 mg. KCN. At F, 1 mg. glutamate and 0·15 mg. KCN. At G, 1 mg. α-ketoglutarate and 0·15 mg. KCN. At H, 1 mg. fumarate and 0·15 mg. KCN.

Potassium cyanide, sodium azide, sodium iodoacetate, *p*-chloromercuribenzoate, sodium monofluoroacetate, sodium thiocyanate were added to the bath in place of reserpine or hydrallazine using the same time cycle[2].

To render the tissue anoxic, the bicarbonate-free Tyrode's solution was first of all boiled to drive off dissolved gases. It was cooled under a mixture of 95 per cent N_2/5 per cent CO_2, and the bicarbonate was added to the cooled solution. The final pH of the Tyrode's solution was 7·6 to 7·8. During the experiment the same gas mixture replaced oxygen in the bath fluid. The tissue was kept under these conditions for 30 minutes before any drugs were added.

RESULTS

The results of tests made on the activity of certain intermediates of carbohydrate, fat and protein metabolism on hydrallazine and reserpine antagonism to contractions induced by ACh (0·1 ng. to 2·0 μg.), Ad

(10·0 ng. to 5·0 μg.), NA (10·0 ng. to 5·0 μg.) 5-HT (40·0 ng. to 3·0 μg.), Hm (0·1 to 5·0 μg.) are shown in Tables I and II. The ability of the added compound to antagonise hydrallazine depression of contractile responses is expressed as the average inhibition per cent of the contractions induced by the stimulant drug. This approximate figure is calculated as follows:

TABLE I

ACTIVITY OF COMPOUNDS TESTED FOR ANTAGONISM TO THE ACTIONS OF HYDRALLAZINE AND RESERPINE ON HORSE CAROTID ARTERY STRIPS

Stimulant drug	Hydrallazine				Reserpine	
	ACh		Ad		ACh	
		Protection per cent		Protection per cent		Protection per cent
Intermediates of—						
(a) *Carbohydrate metabolism:*						
Glucose-1-phosphate	0	0	+	+28	*	
Fructose-1:6-diphosphate	0	0	0	−15		
Glucose-6-phosphate	0	0	++	+75		
Fructose-6-phosphate	+	+24	++	+43		
3-Phosphoglycerate	+	+29	++	+54		
Pyruvate	++	+78	++	+72	+	+12
Succinate	++	+58	++	+42	+	+35
Fumarate	++	+60	0	+10	*	
Citrate	0	+5	0	0		
*cis*Aconitate	0	−40	++	+50		
*iso*Citrate	0	0	+	+18		
α-Ketoglutarate	++	+51	++	+55		
Maleic acid	0	−50	0	−100		
Oxaloacetate	+	+26	++	+60		
Oxalosuccinate	+	+27	+	+19		
(b) *Fat metabolism:*						
3-Hydroxybutyrate	+	+17	++	+57	*	
Propionate	++	+37	++	+58		
(c) *Protein metabolism:*						
Glutamate	++	+35	++	+58	*	
(±)-Alanine	++	+38	++	+53		
(±)-Leucine	0	0	0	0		
(d) *Sulphydryl compound:*						
Glutathione	0	0	0	0	*	
(e) *Other compound:*						
Malate	+	+23	0	0	*	

0 = No activity or antagonism. + = Some activity. ++ = Marked activity.
* Experiments could not be done.

The height of the control contraction (A) is measured and also that of the contraction after addition of the antagonist (B). When there is complete recovery to the control level, or to a reproducible level, which may be slightly greater or less than the original control, the height of the contraction after the addition of antagonist with intermediary metabolite is measured (C). The protection per cent was then calculated,

$$\frac{C - B}{A - B} \times 100 = \text{protection per cent.}$$

Values from all experiments using the same combination of spasmogen, antagonist and the intermediary metabolite have been averaged to obtain these figures. Typical experiments are shown in Figures 1 and 2.

There is some parallelism between results obtained with ACh and Ad (Table I). The most active compounds when selected for use with NA,

5-HT and Hm showed a similar parallelism (Table II) (Fig. 2). Very few experiments could be done with reserpine since it was unusual to get a satisfactory recovery.

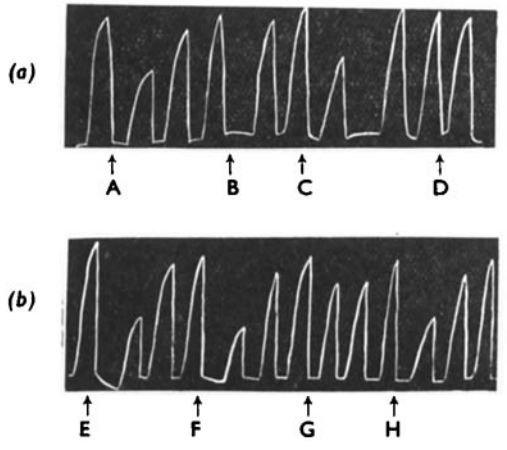

FIG. 4. The effects of succinate, fumarate, pyruvate, butyrate, oxalosuccinate and citrate upon anoxic inhibition of ACh-induced contractions of strips of horse carotid artery. (*a*) All contractions due to 0·04 μg. ACh. At A, anoxia. At B, 1 mg. succinate and anoxia. At C, 1 mg. fumarate and anoxia. At D, 1 mg. pyruvate and anoxia. (*b*) All contractions due to 0·2 μg. ACh. At E, anoxia. At F, 1 mg. butyrate and anoxia. At G, 1 mg. oxalosuccinate and anoxia. At H, 1 mg. citrate and anoxia.

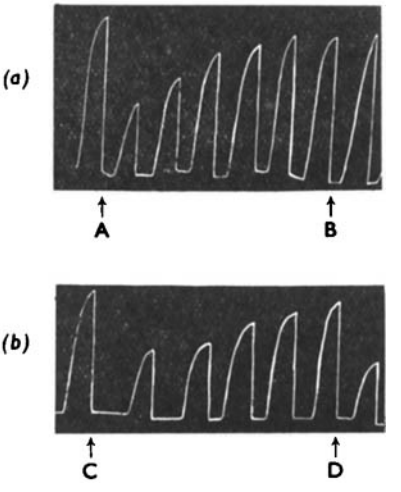

FIG. 5. The inconsistent effects of pyruvate in antagonising azide inhibition of ACh-induced contractions of strips of horse carotid artery. (*a*) All contractions due to 0·1 μg. ACh. At A, 0·4 mg. sodium azide. At B, 1 mg. pyruvate and 0·4 mg. sodium azide. (*b*) All contractions due to 0·4 μg. ACh. At C, 0·8 mg. sodium azide. At D, 1 mg. pyruvate and 0·8 mg. sodium azide.

KCN (0·04 mg. to 0·2 mg.) inhibited ACh-induced contractions of the artery strips. Recovery was generally prolonged or did not take place at all. Table III shows some of the results obtained. There is no close parallelism between the results shown in Table III and those shown in Tables I and II. Some typical experiments are shown in Figure 3.

Anoxia caused loss of smooth muscle tone and diminution of the contractile response to ACh. Recovery of the tissue after supplying oxygen was quite rapid. Table III shows some of the results obtained. A typical experiment is shown in Figure 4.

The results obtained using sodium azide (0·1 to 1·0 mg.) to inhibit ACh-induced contractions of artery strips were too inconsistent for us to be able to draw conclusions. Sodium azide had a prolonged effect and the recovery of response was often very slow (Fig. 5). The effects appeared to be similar to those of KCN.

DISCUSSION

It seems unlikely that hydrallazine and reserpine are acting on specific receptors in arterial smooth muscle. The experiments described were,

therefore, carried out to see whether the effects of hydrallazine and reserpine could be antagonised by supplying a series of known intermediates of carbohydrate, fat and protein metabolism and also to see whether the effects of hydrallazine or reserpine resembled those of anoxia or of known enzyme inhibitors such as azide, cyanide and iodoacetate.

TABLE II

ACTIVITY OF SOME INTERMEDIATES OF CARBOHYDRATE AND PROTEIN METABOLISM IN ANTAGONISING THE ACTIONS OF HYDRALLAZINE ON HORSE CAROTID ARTERY STRIPS

Stimulant drug	Hydrallazine		
	NA	Hm	5-HT
	Protection	Protection	Protection
Intermediates of—			
(*a*) *Carbohydrate metabolism:*			
Pyruvate	++	++	++
Succinate	++	++	++
α-Ketoglutarate	++	++	+
3-Phosphoglycerate	+	+	+
Fructose-6-phosphate	++	++	++
(*b*) *Protein metabolism:*			
Glutamate	+	+	+
(±)-Alanine	0	0	0

0 = No activity or antagonism. + = Some activity. ++ = Marked activity.

The following possibilities come to mind: the compounds may combine chemically with hydrallazine and so partly inactivate it; they may cause liberation of Hm or ACh and hence increase the magnitude of contraction; they may remove calcium ions and thus render the tissue more irritable; or they may act as sources of energy for the tissue. None of the compounds tested caused a direct contraction of the artery strip when added alone to the bath, nor did they potentiate the contractile responses to ACh and Ad. In addition, experiments made in Tyrode's solution containing mepyramine appeared to exclude the possibility of Hm-release. It has already been shown that removal of calcium ions by these compounds is not significant[6]. Citrate is inactive yet this compound is known to remove ionic calcium.

The results shown in Table I indicate that most of the compounds can antagonise hydrallazine depression of ACh and Ad-induced contractions. Why some of them should act and others not, is not clear. It is possible that the inactive compounds do not penetrate the cells or that they are not needed. Most of the active compounds are intermediates of the tricarboxylic acid cycle (although this has not been shown to exist in arterial smooth muscle). 3-Phosphoglycerate, pyruvate, succinate, fumarate, α-ketoglutarate and oxaloacetate are all potent antagonists of hydrallazine-induced inhibition. Citrate, *iso*citrate, *cis*aconitate and maleic acid are inactive or of lower potency. In general, intermediates of glycolysis are less potent, yet when Ad is used both glucose-6-phosphate and fructose-6-phosphate antagonise the actions of hydrallazine. Propionate, glutamate, (±)-alanine, and to some extent 3-hydroxybutyrate also antagonise Ad, whereas (±)-leucine and malate are inactive. The

ability of an amino acid like (±)-alanine to protect against hydrallazine inhibition may be explained by assuming that the arterial smooth muscle can deaminate alanine to its corresponding keto acid, which is pyruvic acid. In the same way the behaviour of oxaloacetate suggests that it may have exerted its effect after decarboxylation to pyruvic acid. It is,

TABLE III

ACTIVITY OF COMPOUNDS TESTED FOR ANTAGONISM TO THE ACTIONS OF KCN AND ANOXIA ON HORSE CAROTID ARTERY STRIPS

Stimulant drug	KCN		Anoxia	
	ACh		ACh	
		Protection per cent		Protection per cent
Intermediates of—				
(a) *Carbohydrate metabolism:*				
Glucose-1-phosphate	0	+10	0	0
Fructose-1 : 6-disphosphate	0	0	0	0
Glucose-6-phosphate	0	0	++	+40
Fructose-6-phosphate	+	+30	++	+45
3-Phosphoglycerate	0	0	+	+25
Pyruvate	++	+68	++	+74
Succinate	+	+16	++	+68
Fumarate	0	0	++	+54
Citrate	0	−70	0	−50
*cis*Aconitate	0	−50	0	−80
*iso*Citrate	0	−50	0	0
α-Ketoglutarate	++	+70	+	+25
Oxaloacetate	0	−55	0	0
Oxalosuccinate	+	+22	++	+44
(a) *Fat metabolism:*				
3-Hydroxybutyrate	0	0	0	0
Propionate	+	+20	0	0
(c) *Protein metabolism:*				
Glutamate	++	+50	+	+11
(±)-Alanine	++	+85	++	+76
(±)-Leucine	0	−36	0	+6
(d) *Other compound:*				
Malate	0	−50	0	+10

0 = No activity or antagonism. + = Some activity. ++ = Marked activity.

therefore, possible to speculate that hydrallazine is interfering with cellular metabolism—possibly in the tricarboxylic acid cycle and that the more potent compounds are supplying energy requirements or replacing a missing metabolite. Hydrallazine is reported to be an histaminase inhibitor[7,8] but it may also inhibit other enzymes.

The results obtained on anoxic tissues using ACh as the stimulant showed some similarity to experiments in which the antagonist was hydrallazine. Anoxic tissues lost their tone and were less sensitive to ACh but sensitivity was never completely lost. Moreover the recoveries of the anoxic and the hydrallazine-treated tissues were strikingly similar. The type of recovery was quite different from that seen after KCN, sodium azide, sodium iodoacetate or reserpine. In these instances the recovery was very slow or absent. When the results obtained with hydrallazine and those obtained with anoxic tissues are compared a close, but not complete resemblance is seen. Thus hydrallazine may alter or interfere with cell metabolism in a somewhat similar manner to oxygen lack.

KCN and sodium azide yielded quite a different picture. Sodium azide caused a pronounced and prolonged inhibition of ACh-induced

contractions but the compounds added had irregular effects and reproducible results could not be obtained. KCN also caused prolonged inhibition of ACh-induced contractions. The effects were antagonised by fructose-6-phosphate, pyruvate, α-ketoglutarate, glutamate, (±)-alanine and less strongly by succinate and propionate. A number of compounds known to combine with sulphydryl groups were tested to see whether their effects were similar to those of hydrallazine. None of these caused inhibition of ACh or Ad-induced contractions. Glutathione added before hydrallazine did not show a protecting effect.

Reserpine caused what was virtually an irreversible effect showing a remarkable affinity for the tissue. This may partly explain the prolonged hypotensive effects of this drug in man.

A few experiments were made with sodium thiocyanate instead of hydrallazine but this compound did not antagonise ACh-induced contractions of artery strips.

Acknowledgements. We are indebted to Dr. C. Dale Falconer of Ciba Laboratories Ltd., for supplies of hydrallazine and reserpine. We are also very grateful to W. C. Hodgkinson Ltd., Glasgow, for supplying us with fresh carotid arteries and to Miss Sheena MacPhee for technical assistance.

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