Inactivation of some vasoconstrictor agonists by saphenous vein strips of the dog*

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The rate of relaxation of aortic strips exposed to sympathomimetic amines and immersed in oil has been described by Kalsner & Nickerson (1968) as a valid and sensitive index of the concentration of agonist at the biophase. It has since been used mainly in studies dealing with the inactivation of catecholamines in some vascular structures. We have examined the technique to ascertain if it could be used in studies with drugs other than catecholamines and to gain information about the termination of action of biogenic substances like 5-HT, prostaglandin $F_{2\alpha}$, histamine and acetylcholine.

Segments of both lateral saphenous veins were obtained from mongrel dogs anaesthetized with pentobarbitone 30 mg kg^{-1} and helically cut strips were prepared as described by Guimarães & Osswald (1969). These were suspended in 20 ml organ baths containing Krebs-Henseleit solution, at 37 °C and bubbled with 95% O_2 + 5% CO_2. After addition of a suitable dose of vasoconstrictor drug, and the contraction had reached steady-state (after 8–10 min of exposure), the bathing solution was replaced by warm and oxygenated mineral oil (Kalsner & Nickerson 1968). The time needed for 50% relaxation (T50) and the influence exerted on this parameter by drugs blocking different transport systems or metabolic pathways were determined as described by Osswald et al (1971). Cocaine (12 μM) and deoxycortone (60 μM) were added to the bath 20 to 30 min before the agonist; iproniazid (0·7 mM) was kept in the bath for 30 min and then washed out (6 times) during the next 30 min.

The results obtained are shown in Table 1. It is apparent that histamine and prostaglandin $F_{2\alpha}$, in the concentrations used, depend largely on diffusion for the termination of their actions, whereas for the other agents efficient inactivating mechanisms are present in the venous strip.

To gain further information on these mechanisms, the influence of appropriate drugs was studied, and the results obtained are presented in Table 2. This shows the factors of prolongation of the T50 in oil, due to the influence of the different drugs. Phenylephrine (lower concentration) and dopamine appear to be subject to both neuronal and extraneuronal uptake, as shown by the influence of the respective blocking drugs, cocaine and deoxycortone. However, 5-HT (and phenylephrine in the higher concentration) is markedly affected by cocaine, but not by deoxycortone and thus is mainly inactivated by neuronal uptake and subsequent deamination by MAO. The combination of cocaine with deoxycortone had roughly additive effects (except with dopamine, where the effect was supra-additive). Oxidative deamination by MAO is clearly the most important metabolic pathway for the amines studied and probably the only one playing any role with phenylephrine, which is in good agreement with data obtained from the rabbit aorta by Kalsner & Nickerson (1968). Intraneuronal MAO is the most important intervening enzyme, as shown by the overlap occurring between iproniazid and cocaine (the combination of the two drugs causes infra-additive effects, especially in the case of 5-HT). An example of the way the results were obtained is illustrated in Fig. 1.

With acetylcholine, physostigmine (4×10^{-7} M) prolonged the T50 in oil by a factor of 5·7 ± 0·8 (n = 4).

We conclude that the method is adequate for the study of the inactivation of constrictor agonists,

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Table 1. Influence of drugs on the time for 50% relaxation (T50) of the saphenous vein strip in oxygenated mineral oil.

Molar concn	Contraction*	T50 in oil (min)	T 50 oil / T50 Krebs	n
Phenylephrine				
$2·0 \times 10^{-6}$	53·6 ± 1·4	3·5 ± 0·3	4·2 ± 0·7	15
$6·0 \times 10^{-6}$	79·6 ± 1·8	16·8 ± 2·0	7·1 ± 0·6	14
Dopamine				
$1·0 \times 10^{-5}$	73·9 ± 1·5	3·4 ± 0·2	2·3 ± 0·3	25
5-HT				
$1·0 \times 10^{-6}$	65·2 ± 1·9	6·4 ± 0·5	2·7 ± 0·2	20
Histamine				
$4·3 \times 10^{-4}$	40·0 ± 2·4	>60	>75	6
Acetylcholine				
$2·2 \times 10^{-4}$	57·8 ± 3·9	10·9 ± 2·6	5·1 ± 0·8	9
PG F2α				
$2·8 \times 10^{-6}$	63·2 ± 6·5	>60	>16	5

* Contraction as % of the maximal contraction caused by noradrenaline.

Table 2. The influence of cocaine (12 μM), deoxycortone (60 μM) or iproniazid (0·7 mM) on the effects of phenylephrine, dopamine and 5-HT on the saphenous vein strip.

	Factors of prolongation of T50*				
Molar concn Cocaine		Deoxycortone	Cocaine + deoxycortone	Iproniazid	Iproniazid + cocaine
Phenylephrine					
$2·0 \times 10^{-6}$	2·6 ± 0·5	2·2 ± 0·2	5·5 ± 0·5	>30	—
$6·0 \times 10^{-6}$	2·0 ± 0·2	1·3 ± 0·1**	3·1 ± 0·3	>12	—
Dopamine					
$1·0 \times 10^{-5}$	1·4 ± 0·2**	2·0 ± 0·2	4·8 ± 0·6	5·6 ± 0·7	6·0 ± 1·2
5-HT					
$1·0 \times 10^{-6}$	3·2 ± 0·3	1·4 ± 0·1**	5·4 ± 0·4	4·6 ± 0·7	9·4 ± 1·6

n = 6 to 8 for each experimental condition.
* T50 in oil after drug treatment/T50 in oil under control conditions.
** N.S.

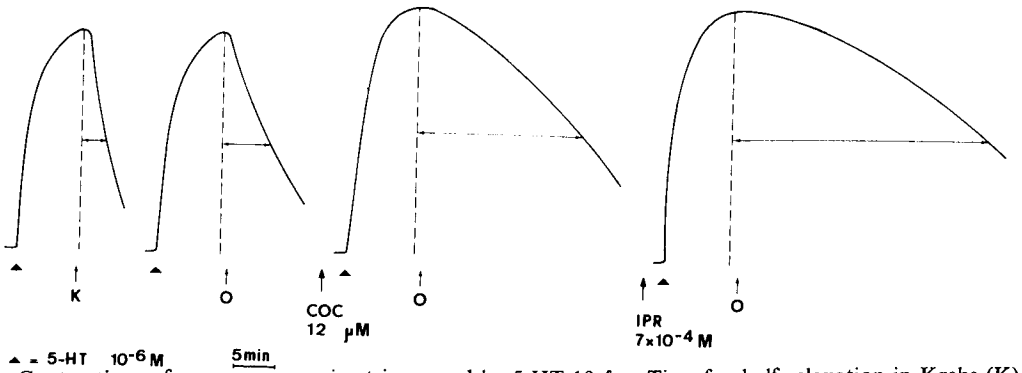

FIG. 1. Contractions of a saphenous vein strip caused by 5-HT 10^{-6} M. Time for half-relaxation in Krebs (K) and in oil (O) is represented by the horizontal arrows Iproniazid (IPR) slows the relaxation down much more than cocaine (COC); see Table 2.

with the reservation that in some cases (e.g. histamine) the concentrations needed to cause reasonable contractions may be too high and exceed the metabolizing capacity of the strip. It is clear that diffusion is the major mechanism for the termination of the action of histamine and prostaglandin $F_{2\alpha}$, whereas 5-HT, phenylephrine and dopamine (in decreasing order) are essentially taken up by adrenergic nerve terminals. Oxidative deamination is responsible for the final metabolic fate of 5-HT and phenylephrine, and hydrolysis by cholinesterase for acetylcholine. Extraneuronal uptake (and, eventually, subsequent metabolism) appears to play a significant role only for dopamine (and of low concentrations of phenylephrine).

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Effects of urogastrone on mechanical activities of the stomach and intestine of guinea-pig

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Urogastrone has been established as an inhibitor of gastric secretion (Lawrence et al 1971; Gregory 1970) and has been used clinically in gastric ulcer. However, the effects of urogastrone on the mechanical activity of smooth muscles remain obscure. Therefore I have examined the mode of action of urogastrone on gastrointestinal smooth muscle.

Female Hartley guinea-pigs, 300 to 400 g, were killed by a blow on the head, the ileum was isolated and a piece (3 to 4 cm) taken from the middle ileum was suspended in a 20 ml organ bath containing a physiological solution containing (g) NaCl 9·0, KCl 0·4, $CaCl_2$, 0·2, $MgCl_2$ 0·1, $NaHCO_3$ 1·0 and glucose 1·0 g in 1 litre) kept at 32 °C and gassed with 5% CO_2 in oxygen. Responses to drugs were recorded isotonically under a tension of 0·5 g. Drugs were added cumulatively to the bath fluid to obtain dose-response curves of the drugs in all experiments except those in Fig. 2. In some experiments electrical stimulation was according to Paton (1957). The electrodes were made of platinum and the intraluminal electrode was the anode. Rectangular pulses of 0·1 ms duration were used at frequencies of 0·1 and 50 Hz (for 2 s at intervals of 2 min) and strength sufficient to give a maximal response. The responses of ileum to electrical stimulation were recorded isometrically with an initial tension of 1·0 g.

The effect of urogastrone on the spontaneous movements of the stomach in situ was tested in the guinea-pig. A male guinea-pig (400 to 500 g) laparotominized under sodium pentobarbitone (30 mg kg^{-1} i.p.) had a rubber microballoon implanted into the muscle layer of pyloric antrum. At least three days after the implantation and a fast of 24 h, the experiments were begun in the conscious animal. The internal pressure of the balloon was recorded by a low pressure transducer (Nagasawa et al 1974). Urogastrone used was kindly supplied from Tobishi Pharmaceutical Co. Ltd., Japan.

Dose response curves for acetylcholine, histamine and $BaCl_2$ were not influenced by urogastrone 3×10^{-5} g ml^{-1} but were slightly potentiated by 10^{-4} g ml^{-1} (data not shown). The response of the ileum to electrical stimulation at 0·1 Hz was inhibited by urogastrone (10^{-5} to