Effects of glyceryl trinitrate on the aortic strip of rabbits

SIR,—We have investigated the effect of glyceryl trinitrate in relaxing the vascular spasm produced by a maximal dose of noradrenaline in various ionic media, under different conditions of temperature and hydrogen ion concentration.

The experimental technique for the aortic strip preparation was similar to that previously described (Lorenzetti, Tye & Nelson, 1966). All solutions were in 0·9% sodium chloride solution with the pH adjusted to 7·3 ± 0·1. Bathing media were prepared with triple glass distilled water as in Table 1. Drug solutions were prepared daily and kept in an ice bath during use. All doses were added to the bath in a volume of 0·05 ml. Two to four doses of agonist were used until uniform maximal contraction was observed, with 30-min wash periods between doses. When uniform contractions were assured, the agonist was introduced and the tissue allowed to attain peak contraction, passing through the fast and slow phase of contraction (Brodie, Bohr & Smit, 1959). At the height of contraction glyceryl trinitrate was introduced, starting at 4×10^{-6} μM/ml; subsequent increasing log doses of glyceryl trinitrate were tested after washing the strip for 30 min. A plot of 3 to 5 points on the curve of log concentration against response was made. From this graph the relaxant dose 50 (RD 50), i.e., the dose of glyceryl trinitrate needed to reduce the contraction induced by the agonist by 50%, was determined.

Raising or lowering the pH of the bathing media with the temperature constant at 37° decreased the sensitivity of the preparation (Table 2) to glyceryl trinitrate. Lowering of the temperature below 36° decreased the sensitivity of the preparation to glyceryl trinitrate. But in decreasing the temperature toward 36°, the

TABLE 1. COMPOSITION OF BATHING MEDIA USED FOR AORTIC STRIP EXPERIMENTS (IN MMOLE/LITRE) AND CONCENTRATION OF GLYCERYL TRINITRATE REQUIRED TO REDUCE THE MAXIMUM CONTRACTION TO 0·0059 μMOLE NORADRENALINE BY 50% (RD50) IN THESE SOLUTIONS

Solution	Norm Krebs HCO_3	Ca-Ca-Free	High Ca	K Free	High K	Mg-Free	High Mg	High Barium	Mg-Ca Free
Sodium chloride	118·5	120·0	110·0	123·0	110·0	120·0	115·0	110·0	128·0
Potassium chloride ..	4·8	4·8	4·8	—	13·3	4·8	4·8	4·8	4·8
Calcium chloride.$2H_2O$	1·9	—	6·9	1·9	1·9	1·9	1·9	1·9	—
Potassium dihydrogen phosphate ..	1·2	1·2	1·2	—	—	1·2	1·2	1·2	—
Sodium dihydrogen phosphate ..	—	—	—	1·2	1·2	1·2	—	—	—
Magnesium sulphate·$7H_2O$	1·2	1·2	1·2	1·2	1·2	—	5·2	—	—
Sodium bicarbonate	25·0	25·0	25·0	25·0	25·0	25·0	25·0	25·0	—
Glucose.. ..	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0
Trizma base (Tris) ..	—	—	—	—	—	—	—	—	40·0
Barium chloride	—	—	—	—	—	—	—	2·5	—
Glyceryl trinitrate RD 50 (µmole/ml ± s.e.)	0·0064 ±0·0009 (16)*	0·0037 ±0·0012 (8)	† (9)	0·0035 ±0·0015 (12)	0·00061 ±0·00008 (12)	† (9)	0·004 ±0·001 (9)	† (18)	† (9)

Parameters: ionic strength range, 0·168 to 0·174: osmolarity range, 314·5 to 317·5 milliosmoles; pH range, 7·28 to 7·44, unless indicated. The solutions were gassed for 1 hr with 100% carbon dioxide before adding to the reservoir, then gassed with 95% oxygen-5% carbon dioxide throughout the experiment. All reagents are analytical grade.

* No. of experiments.

† Relaxation not greater than 25%.

TABLE 2. CONCENTRATION OF GLYCERYL TRINITRATE REQUIRED TO REDUCE THE MAXIMUM CONTRACTION[1] BY 50% (RD 50 μMOLE/ML ± S.E.) AT VARIOUS TEMPERATURES AND HYDROGEN ION CONCENTRATION

Test situation[1]	No. of experiments	RD 50 (μmole/ml ± s.e.)
Temp = 37° ± 1° pH = 7·35 ± 0·5	16	0·0064 ± 0·0009
Temp = 36° ± 1° pH = 7·35 ± 0·5	18	0·0012 ± 0·0003
Temp = 33° ± 1° pH = 7·35 ± 0·5	8	0·0181 ± 0·0017
Temp = 27° ± 1° pH = 7·35 ± 0·5	9	0·35 ± 0·0025
Temp = 37° ± 1° pH = 6·9 ± 0·5	9	0·011 ± 0·0008
Temp = 37° ± 1° pH = 7·75 ± 0·5	9	0·013 ± 0·0059

[1] Glyceryl trinitrate administered after peak response to 0·0059 μmole of noradrenaline.

RD 50 for glyceryl trinitrate was significantly lower ($P < 0·001$) than at 37°. This temperature difference gave a consistantly optimal response to the effect of glyceryl trinitrate against the maximum contraction induced by noradrenaline. At 27° the tissue changed in physical appearance from its normal pink colour to white. Temperatures of 40 and 42° made the tissue unresponsive. Below 36° the response to noradrenaline consisted mostly of the slow phase of response and required 7 to 9 min longer than the usual 2 to 4 min to attain its peak.

Removal of all traces of calcium from the Krebs-bicarbonate media decreased the contraction induced by noradrenaline by more than 50% (Table 1). The absence of calcium also increased the sensitivity of the aortic tissue to glyceryl trinitrate (0·0064 versus 0·00037 μM/ml). The only other altered electrolyte medium which required less glyceryl trinitrate to produce a 50% response was the high (13·3 mM) potassium medium. With both the high potassium and high calcium media, the contractile response to noradrenaline was increased by 30 to 40%.

The dual contractile response of the aorta consists of a fast (F) response followed by a slower yet sustained contraction, referred to as the slow (S) response. The F-response occurs within the first 2 min while the S-response may extend another 1½–2 min. The S-response was eliminated by absence of potassium or magnesium or by the high calcium medium while the F-response was little altered by these conditions. The differentiation of the S- and F-response which occurred with no magnesium or high calcium was seen consistently in 18 aortic strip preparations.

Strips which were equilibrating in high K^+, Ba^{2+} or Ca^{2+} Krebs-bicarbonate media consistently displayed a more rapid increase in tone. Absence of these ions as well as high Mg^{2+} Krebs-bicarbonate caused a rapid loss in tone during the equilibration period as well as a 10 to 15% decrease in the noradrenaline response. Absence of calcium gave the greatest sensitivity to glyceryl trinitrate (Table 1), although the contraction induced by noradrenaline was reduced 12 to 14%. High Mg^{2+} (5·2 mmole) and absence of K^+ required more glyceryl trinitrate to produce a 50% relaxant effect. The effect of altering other ions shown in Table 1 was not determinable because of the insensitivity of the tissue. High concentration of Ca^{2+}, K^+, Ba^{2+}, as well as absence of Mg^{2+} potentiated the noradrenaline response 30 to 45%. Much of the potentiation occurred in the S-response with some enchancement appearing in the F-response also. Absence of Ca^{2+} and Mg^{2+} caused a reduced response to noradrenaline of the F-response only, in seven out of nine trials. A reduction in the S-response

was also seen with the calcium free Krebs-bicarbonate. It seems that glyceryl trinitrate relaxation response on vascular muscle may be influenced by the ionic media in isolated tissue studies. Possibly muscular relaxation induced by glyceryl trinitrate is accompanied by ion fluxes.

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Effect of adrenal demedullation and adrenalectomy on amphetamine toxicity in isolated and aggregated mice

SIR,—Amphetamine toxicity is greater in aggregated than in isolated mice (Thiessen, 1964), and heightened adrenal secretory activity (medullary or cortical, or both) in aggregated mice has been implicated as a causative factor.

D'Arcy & Spurling (1961) found that amphetamine toxicity in isolated mice was increased by pretreatment with cortisol or corticotrophin. Moreover, Weiss, Laties & Blanton (1961) reported that adrenalectomy reduced the enhanced toxicity of amphetamine in isolated mice subjected to unavoidable foot-shock. Foot-shock also increases amphetamine toxicity in individual as well as aggregated mice; moreover, the events leading to death in amphetamine-treated, shocked, isolated mice appeared to be the same as those observed in aggregated mice treated with this drug (Weiss & others, 1961; Askew, 1962). Most recently, Richards, Nicol & Young (1966) reported that adrenalectomy reduced the enhanced toxicity of desoxyephedrine in aggregated mice. In contrast, Mennear & Rudzik (1965) observed that amphetamine toxicity in aggregated mice was not altered by adrenalectomy. We have now made some experiments designed to determine the significance of the adrenal in the "amphetamine aggregation effect".

Novice, male, albino mice of a random bred Swiss strain (Maxfield; Cincinnati, Ohio) were injected intraperitoneally with an aqueous solution of (+)-amphetamine sulphate (1 ml/100 g body wt), and were either isolated or aggregated (3 per cage) in metal cages (7 × 7 × 7·5 cm), one side of which was wire mesh to permit observation. Aggregated mice that died during the 3 hr observation period were replaced by untreated mice to maintain aggregation. Ambient temperature was 24 ± 1°.

To evaluate the significance of the adrenal medulla in the amphetamine aggregation effect, (+)-amphetamine toxicity was measured in demedullated, sham-operated and non-operated mice. Enucleation was done under ether anaesthesia via bilateral incisions in the lumbar musculature. Each adrenal capsule was incised and the medulla with most of the attached cortical parenchyma gently squeezed out with small forceps. Histology showed regeneration of the cortex, but not the medulla, to take place within 21 days. The adrenal capsule was not incised in sham-operated mice; non-operated mice remained caged throughout the operative period. Post-operatively, mice were housed in their home cages (45 × 24 × 12 cm) in groups of 15 for not less