EFFECT OF METABOLIC POISONS AND DIGOXIN ON THE ADRENERGIC NEURONE BLOCKING ACTION OF GUANETHIDINE

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Summary : The adrenergic neurone blocking action of guanethidine in isolated rabbit ileum preparation (Finkleman) was neither prevented nor reversed by either iodoacetate or dinitrophenol. However iodoacetate and dinitrophenol together partially prevented but did not reverse the neurone blocking action of guanethidine. Digoxin totally prevented but did not reverse the neurone blocking action of guanethidine.

Key Words : Guanethidine Iodoacetate and dinitrophenol Digoxin
Adrenergic neurone blockade

Gulati and Jaykar (7) studied the uptake of guanethidine indirectly by testing the ability of various procedures to prevent or reverse adrenergic neurone blockade in the periarterially stimulated isolated rabbit ileum preparation described by Finkleman (4). They showed that adrenergic neurone blockade was prevented but not reversed by equilibration with guanethidine at low temperature (10°C), in the absence of sodium or in the presence of tetrodotoxin or noradrenaline. It was concluded that the neuronal mechanism concerned with the uptake of noradrenaline could take up guanethidine and that this uptake of guanethidine was energy and sodium dependent. This paper reports the effects of metabolic inhibitors and of digoxin since it is known that digitalis glycosides inhibit the sodium-potassium ATP-ase (12)

METERIALS AND METHODS

Details of the method for setting up Finkleman preparation are described in the earlier paper (7). The relaxant responses of the rabbit ileum to mesenteric nerve stimulation showed variations from preparation to preparation. Therefore, two preparations were set up simultaneously. After eliciting control responses to different frequencies one preparation was treated with the test drugs for a specific period of time. Both the preparations were now exposed to guanethidine (3.3×10^{-6} M) for 10 min. The preparations were washed several times with McEwan's solution (the solution used for bathing) and the responses to nerve stimulation were recorded. The effects of the test drug on responses to nerve stimulation were studied by setting up a third preparation from the same ileum and exposing it to the test drug for a specified time. Guanethidine (3.8×10^{-6} M) produced substantial to complete block of responses of the ileum to periarterial sympathetic nerve stimulation at frequencies ranging from 2 sec to 60 sec. Responses

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to noradrenaline (0.59 x 10^{-8}—0.59 x 10^{-7}M) were unaffected. The blocking action lasted fo more than 3 hr.

RESULTS

Iodoacetate : Exposure of the tissue to iodoacetate (2.7 x 10^{-6}M) for 30 min reduced it tone and pendular movements which did not recover completely even after several washes fo over 2 hr. Responses to nerve stimulation were partially blocked (Fig. 1). Responses to nora drenaline (0.59 x 10^{-8}—0.59 x 10^{-7}M) were not blocked.

In control preparations guanethidine produced complete adrenergic neurone blockade a all the frequencies. Iodoacetate (2.7 x 10^{-6}M) kept in the bath for 20 min before exposing th tissue to guanethidine did not prevent adrenergic neurone blockade due to guanethidine (Fig. 1 In two experiments exposure of the preparation to iodoacetate (2.7 x 10^{-6}M) for 30 min afte guanethidine had completely blocked responses to nerve stimulation, did not reverse the adre nergic neurone blocking action of guanethidine.

Dinitrophenol : Exposure of preparations to dinitrophenol (0.5 x 10^{-3}M) for 30 mi reduced the tone and abolished the pendular movements. Responses to nerve stimulatio were abolished. Responses to exogenous noradrenaline (0.59 x 10^{-8}—0.59 x 10^{-7}M) were no affected. Soon after a few washes, the tone and the pendular movements and responses t nerve stimulation were restored to normal (Fig. 1).

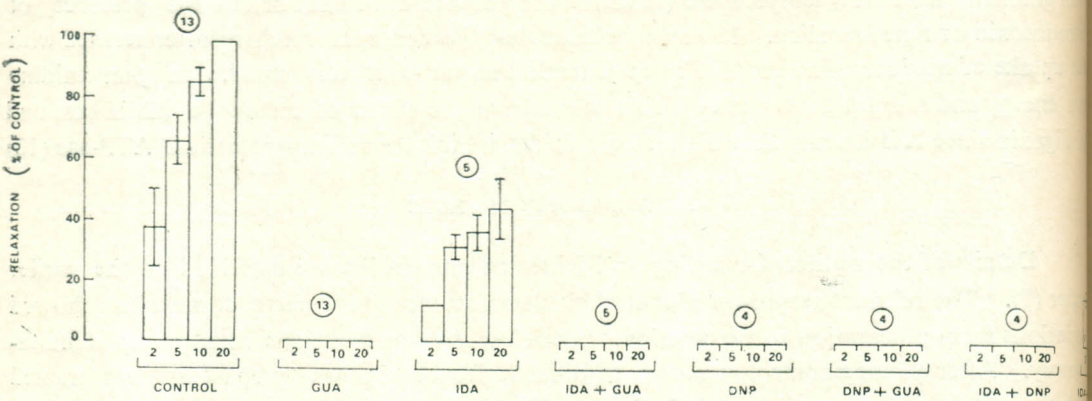

Fig. 1: Response of Finkleman preparation to periarterial nerve stimulation at different frequencies, e pressed as % of controls. From left to right the first panel shows control and second, third, fifth an seventh panels show responses after exposure of the preparations to guanethidine (3.3x10^{-6} M) for 10 mi iodoacetate (2.7x10^{-6} M) for 30 min, dinitrophenol (0.5x10^{-3} M) for 30 min and iodoacetate (2.7x10^{-6} M and dinitrophenol (0.5x10^{-3} M) for 30 min respectively. Responses shown in the fourth, sixth and eigh panels were obtained by exposing the preparations to iodoacetate (2.7x10^{-6} M) for 20 min, dinitrophen (0.5x10^{-3} M) for 20 min and iodoacetate (2.7x10^{-6} M) and dinitrophenol (0.5x10^{-3} M) for 20 min re pectively followed by further exposure to guanethidine (3.3.x10^{-6} M) for 10 min. Vertical lines indic standard errors. Encircled figures above each panel indicate the number of observations.

In control preparations guanethidine totally blocked responses to nerve stimulation at all the frequencies. Exposure of the preparation to dinitrophenol (0.5×10^{-3}M) for 20 min before adding guanethidine failed to prevent the adrenergic neurone blocking action of guanethidine (Fig. 1). Dinitrophenol (0.5×10^{-3}M) kept for 30 min in the bath failed to reverse the adrenergic neurone blocking action of guanethidine (2 experiments).

Iodoecetate and dinitrophenol : When the prepatations were exposed to diooacetate (2.7×10^{-6}M) and dinitrophenol (0.5×10^{-3}M) for 20 min they were relaxed, the pendular movements were abolished and responses to nerve stimulation were totally blocked (Fig. 1). Responses to exogenous noradrenaline (0.59×10^{-8}—0.59×10^{-7}M) were not affected. After several washes the tone and pendular movements were only partially restored, but responses to nerve stimulation continued to remain blocked for over 2 hr.

In control preparations guanethidine produced total adrenergic neurone blockade. Iodoacetate (2.7×10^{-6}M) and dinitrophenol (0.5×10^{-3}M) placed in the bath for 20 min before exposing the tissue to guanethidine partially prevented the adrenergic neurone blocking action of guanethidine (Fig. 1). Combined treatment with iodoacetate (2.7×10^{-6}M) and dinitrophenol (6.5×10^{-3}M) for 30 min failed to reverse the adrenergic neurone blocking action of guanethidine (2 experiments).

Digoxin : Fig. 2 summarises the data.

Following exposure of the tissue to digoxin (3.0×10^{-6}M) for 20 min and washing, the tone and pendular movements were reduced and responses to nerve stimulation were blocked. The action of digoxin lasted for more than 2 hr. Responses to exogenous noradrenaline (0.59×10^{-8}—0.59×10^{-7}M) were not affected.

In control preparations guanethidine substantially blocked responses to periarterial nerve stimulation. In test preparations digoxin (3.0×10^{-6}M) was placed in the bath for 10 min followed by further exposure of the tissue to guanethidine. The adrenergic neurone blocking action of guanethidine was totally prevented.

In the control experiments described above, where the tissue was exposed to guanethidine it was treated with digoxin (3.0×10^{-6}M for 20 min) after eliciting a panel of responses to different frequencies of nerve stimulation. The tissue was now washed five to six times and the responses to different frequencies were re-elicited. Responses to nerve stimulation were not significantly ($p > 0.05$) different from the control guanethidine responses. Thus digoxin could not reverse the adrenergic blocking action of guanethidine.

DISCUSSION

Interference with energy metabolism reduces the uptake and retention of noradrenaline in the isolated tissues (3,6,13). The uptake of noradrenaline by the guinea pig isolated left atrium

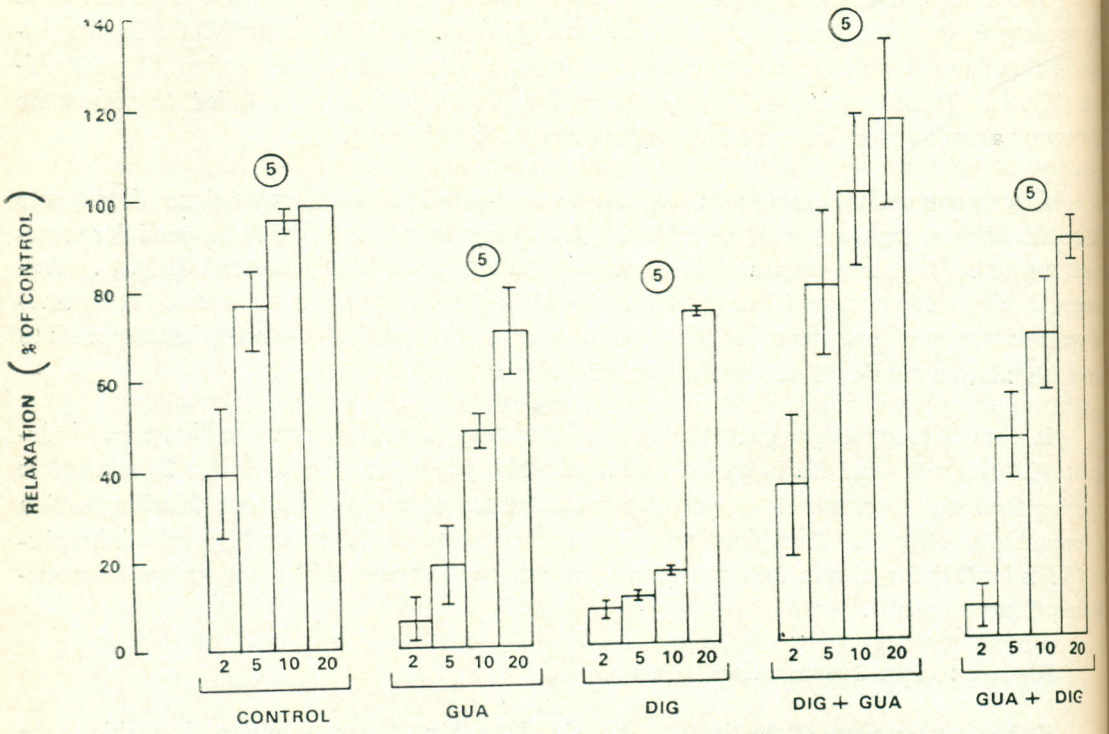

Fig. 2 : Responses of Finkleman preparations to periarterial nerve stimulation at different frequencies, expressed as % of controls. From left to right the first panel shows control responses; second and third panels show responses after exposure of the preparations to guanethidine ($3.3x10^{-6}$ M) for 10 min and digoxin ($3.x10^{-6}$ M) for 20 min respectively; responses shown in the fourth panel were obtained after exposing the preparations to digoxin ($3.0x10^{-6}$ M) for 10 min followed by exposure to gunethidine ($3.3.x10^{-6}$ M) for 1 min in the pressence of digoxin and several wash-outs; after eliciting responses shown in the second panel the preparations were treated with digoxin ($3.0x10^{-6}$ M) for 20 min, washed and the responses re-elicited (fifth panel). Vertical lines indicate standard errors. Encircled figures above each panel indicate the number of observations.

is not exclusively dependent on carbohydrate metabolism, and the energy required for the uptake can also be provided by aerobic oxidation of noncarbohydrate endogenous substrates (13). In the present study the metabolic inhibitors, iodoacetate or dinitrophenol individually did not prevent the adrenergic neurone blockade due to guanethidine but together were able to prevent it partially. This agrees with the reported metabolic requirements for the uptake of the noradrenaline (13) and α- methyl-noradrenaline (8,9). The failure of iodoacetate and dinitrophenol to completely prevent the adrenergic neurone blocking action of guanethidine may partly be due to its passive diffusion into the adrenergic neurone. Alternatively the persistent adrenergic neurone blocking action of iodoacetate may have masked the preventing action of metabolic inhibition on adrenergic neurone blockade by guanethidine. When iodoacetate and dinitrophenol either separately or in combination were added to the bath after exposure of the tissue to

guanethidine, the adrenergic neurone blocking action of guanethidine was not affected at all. It may, therefore, be justifiably concluded that the uptake of guanethidine by the adrenergic neurone is in part energy-dependent.

Digoxin prevented the neurone blocking action of guanethidine. Dengler *et al* (3) first demonstrated that ouabain could block the accumulation of noradrenaline by brain and heart slices. Ouabain exerts this effect by inhibiting the transport of noradrenaline across the cell membrane rather than by acting on storage granules (1). Kirpekar and Wakade (10) showed that cuabain can produce marked interference with the uptake of noradrenaline by the perfused spleen of the cat. Ouabain blocks the transport of 5-HT into platelets by an action on the cell membrane (11). The amine transport system is blocked indirectly through the inhibition of sodium-potassium ATP-ase (12). An ion-coupled transport such as that described for noradrenaline (2,5,10) and suggested for guanethidine (7) finds further support from the present data on the preventing action of digoxin of the adrenergic neurone blocking action of guanethidine since the sodium-potassium dependent ATP-ase which is crucial in determining extracellular sodium concentration is blocked by digitalis glycosides (12). It appears that sodium ions play an obligatory role.

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