INHIBITORY ACTION OF AMINOGLYCOSIDE ANTIBIOTICS ON THE ELETRICALLY INDUCED RELEASE OF OPOID PEPTIDES IN THE GUINEA-PIG ILEUM *IN VITRO*

M MAHMOUDIAN, A. R. DEHPOUR AND M. EMAMI

Department of Pharmacology and Experimental Medicine,
School of Medicine,
University of Tehran, Tehran, Iran

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Summary : The effect of aminoglycoside antibiotics upon the electrically induced release of endogenous opoid peptides from the gninea-pig ileum was studied *in vitro*. Stimulation of guinea pig ileum at 10 Hz in normal Tyrode solution resulted in the naloxone sensitive depression of the twitch contractions of this muscle. Addition of aminoglycosides during 10 Hz stimulation diminished this naloxone sensitive depression in a dose dependent manner. IC_{50}s of this effect of streptomycin, neomycin, kanamycin and gentamicin were found to be 2.54, 2.29, 1.36 and 0.7 *mg/ml* respectively. A 3.5 fold increase in the calcium concentration of media during 10 Hz stimulation significantly reversed the effect of aminoglycosides. It is concluded that aminoglycoside antibiotics exert their effect by interfering with trans-membrane movements of calcium at the nerve endings which is required for the electrically induced release of opoid peptides.

Key words: aminoglycoside antibiotics opoid peptides calcium guinea-pig ileum

INTRODUCTION

It has been established that 0.1 Hz stimulation of the guinea-pig ileum induces contractions which are depressed after application of opioid peptides (1) On the other hand, application of 10Hz stimulation results in a naloxone sensitive depression of twitch height which is caused by the release of endogenous opioid peptides in this tissue (5). This depression of twitch contractions by 10Hz stimulation is calcium dependent and markedly reduced by decreasing the calcium concentration of the media (2).

Due to the fact that aminoglycoside antibiotics affect the release of various transmitters at nerve endings by inhibition of Ca^{2+} uptake (4.6), we were encouraged to investigate whether aminoglycosides could also affect the release of endogenous opioid peptides in the guinea-pig ileum preparations. The results are presented in this communication.

MATERIAL AND METHODS

Chemicals : Gentamicin sulphate, kanamycin sulphate, neomycin sulphate and streptomycin sulphate (Pharmaceutical grade) were gifts from Daru Pakhash Co, (Tehran-Iran). Other chemicals were analytical grade.

General procedure : Male guinea-pigs (200-300 *g*) were sacrificed by stunning and bleeding. Pieces of ileum (2-3 *cm* long) were excised and mounted in a 16 *ml* organ bath containing normal Tyrode solution (composition, mM, NaCl 137, KCl 2.7, $CaCl_2$ 1.8, NaH_2PO_4 0.4, $MgCl_2$ 1.5, $NaHCO_3$ 11.9, and glucose 5). The temperature was maintained at 37°C and the solution was bubbled with a 95% 0_2 and 5% CO_2 mixture throughout the experiment. The ileum was stimulated through two platinum ring electrodes with rectangular pulses of 1 msec duration applied at a frequency of 0.1 Hz and supramaximal voltage by Grass Stimulator 44. The isotonic contractions of the muscle were recorded with the aid of a Narco Isotonic Myograph Transducer and Narco Physiograph.

The naloxone sensitive inhibition was elicited by a 5 min stimulation of the same voltage and pulse duration but at a frequency of 10 Hz as described by Puig *et al.* (5). The area of the recorded contributions generated during 5 min by stimulation at 0.1 Hz was measured before (basal response; BR) and immediately after stimulation at 10 Hz (post stimulation response; PSR). The inhibitory response was calculated by subtraction of the PSR from the BR. The percentage inhibitory response (IR) was calculated as (BR-PSR/BR) x 100.

Alternately, a solution of each of aminoglycosides or other chemicals were added to media during the 10 Hz stimulation period. After 10 Hz stimulation period the tissue was washed three times rapidly with fresh Tyrode solution and the response of the ileum at 0.1 Hz stimulation was recorded before.

Antagonism of the inhibitory response, represented as percentage reversal, was calculated at 100x $(\%IR_c - \%IR_d)/(\%IR_c)$, where IR_c is the inhibitory response of the control and IR_d is the inhibitory response in the presence of the drug (5).

High calcium media : A solution of calcium chloride was added to the media at the time of 10 Hz stimulation so that the calcium content of the media changed from 1.8 mM to 6.3 mM either in the absence or presence of gentamicin (0.75 *mg/ml*), kanamycin (1.5 *mg/ml*), neomycin (0.75 *mg/ml*), or streptomycin (0.75 *mg/ml*), and response of the ileum was recorded as above.

RESULTS

Effect of 10 Hz stimulation : The twitch contractions of guinea-pig ileum, which were elicited by stimulation at 0.1 Hz, were markedly inhibited after 5 min stimulation at 10 Hz (panel a, Fig. 1). This inhibition was antagonized by addition of 10^{-6}M naloxone to the bathing media prior to 10 Hz stimulation.

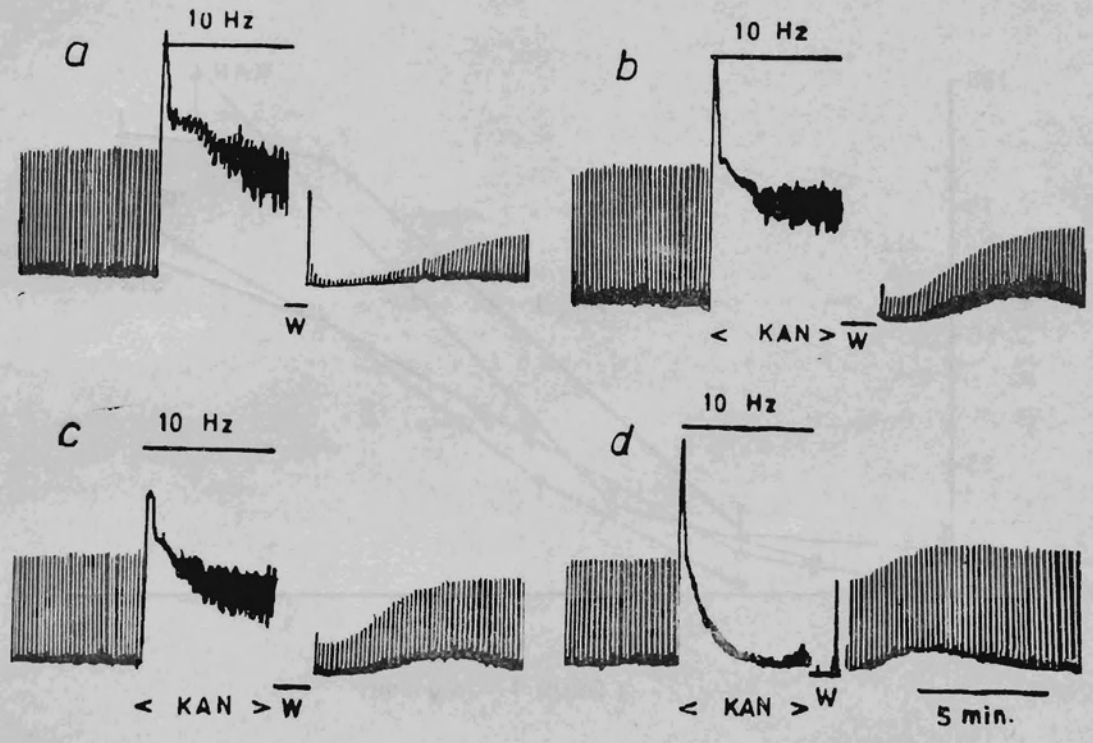

Fig. 1 : The inhibitory action of 10 Hz stimulation on the contractions of the guinea-pig ileum elicited by 0.1 Hz (a), and the reversal of this effect by kanamycin. Kanamycin was added during 10 Hz stimulation at concentration of 0.5 *mg/ml* (b) 1.5 *mg/ml* (c), 6.67 *mg/ml* (d) and washed afterward (w).

Interaction of aminoglycosides with 10 Hz stimulation : The addition of kanamycin during 10 Hz stimulation period resulted in the reduction of the inhibitory effect of 10 Hz stimulation in a dose dependent manner (Figs. 1 and 2). A similar result was obtained for other aminoglycosides, i.e., gentamicin, neomycin and streptomycin, which is shown in Fig. 2. Gentamicin showed the highest potenoy, IC_{50} being 1.6 *mg/ml* followed by kanamycin, neomycin and streptomycin, respectively.

Effect of high calcium media : A 3.5 fold increase in the calcium concentration of the media during 10 Hz stimulation and in the presence of kanamycin reversed the above mentioned effeot of kanamycin, and 10 Hz stimulation in this case resulted in the inhibition of twitch contraction of ileum to an extent comparable to that of control, i.e. in the absence of kanamycin and normal calcium media. Similar results were obtained for other aminoglycosides and are presented in Table I.

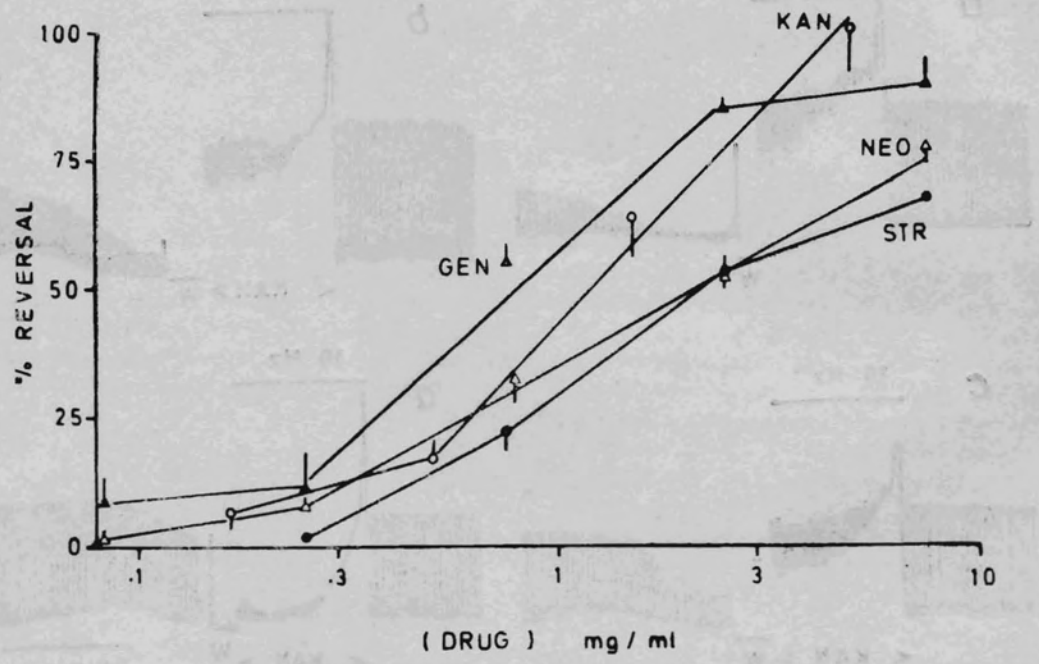

Fig. 2 : Dose-response curves of the reversal effect of various aminoglycosides upon the inhibitory action of 10Hz stimulation on the guinea-pig ileum contractions. Gentamicin (▲——▲), Kanamycin (○——○), neomycin (△——△), and streptomycin (●——●) was added during 10Hz stimulation and the contractions of the ileum was compared prior and after 10 Hz stimulations and the contractions of the ileum was compared prior and after 10 Hz stimulation. The percent of the reversal effect (ordinate) was determined as described in the text. Each point is the mean of at least five experiments. Bars indicate the S.E. of the means.

DISCUSSION

Our results clearly demonstrate that a 10 Hz stimulation of the guinea-pig ileum results in depression of the twitch height in this tissue which is antagonized by naloxone.

This is consistent with the previous observations of Puig *et al.* (5), who demonstrated that the depression of the twitch height of the guinea-pig ileum after 10 Hz stimulation is due to the release of endogenous opioid peptides.

We also have shown that this phenomenon is antagonized dose-dependently when the tissue is treated with aminoglycosides during 10 Hz stimulation.

Taking into consideration that the inhibitory action of 10 Hz stimulation on the guinea-pig ileum can be reversed by lowering the Ca^{2+} concentration of the media (2), and the fact that the same effect has been observed following the application of aminoglycosides, it may be assumed that aminoglycosides exert their effect via an antagonism with Ca^{2+} ions. This hypothesis is further supported by the observation that, it is possible to overcome the effect of aminoglycosides by increasing the Ca^{2+} concentration of the media (Table I).

TABLE I : Effect of aminoglycoside antibiotics upon inhibitory action of 10 Hz stimulation in guinea-pig ileum in different media % of inhibition, mean (±S. E. M.).

	(A) *Drug free normal media*	(B) *Normal media+drug*	(C) *High Ca^{2+} media+drug*	*T-test B vs C*
Ca^{2+} conc. DRUG	1.8 mM	1.8 mM	6.3 mM	
Streptomycin 0.75 *mg/ml*	89.9 (±5.6)	52.3 (±3.2)	76.3 (±12.2)	$P < 0.05$
Kanamycin 1.5 *mg/ml*	88.3 (±4.0)	26.8 (±9.2)	52.0 (±8.9)	$P < 0.05$
Gentamycin 0.75 *mg/ml*	95.7 (±1.3)	42.0 (±12.2)	75.3 (±10.6)	$P < 0.05$
Neomycin 0.75 *mg/ml*	91.0 (±4.2)	32.0 (±6.7)	74.0 (±3.5)	$P < 0.01$

* Each value is the mean of at least four experiments.

The interaction of aminoglycosides with transmembrane movement of Ca^{2+} has been shown in various preparations. For example, streptomycin reduces the uptake and increases the efflux of ^{45}Ca induced in rat isolated superior cervical ganglia by preganglionic nerve stimulation (3).

Considering these observations and our results, it may be concluded that amino-

glycosides exert their effect presumably via an inhibition of Ca^{2+} transmembrane movements which is required for the release of endogenous opioid peptides and thereby inhibit the action of 10 Hz stimulation.

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