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Frequency–Force Relationship on Isolated Rat and Guinea Pig Atria. Effects of Cholinergic and Adrenergic Receptor Antagonists[1] (37971)

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The frequency–force or interval–strength relationship, i.e., the modification of contractile strength of muscle associated with changes in the frequency of contractions, has been described in auricles (1–6) and ventricles (6–9) of several mammalian species, including the human heart (6, 9). Most of these reports agree that the majority of heart preparations respond with a progressively higher contractile tension within certain ranges of increment in the frequency of contractions. This stepwise change in the strength of contraction has been described as: positive staircase, ascending staircase, "treppe," or "Bowditch phenomenon" (9, 10). On the contrary, in some cases, such as in ventricles and auricles from rats (2, 4, 6, 9, 11) or in human heart preparations (6, 9), the frequency–force relationship appears atypical, i.e., the increment in the rate of contractions, within a range comparable to that at which other preparations show a positive inotropic response, results in a decrement of contractile tension. This behavior has been identified under different names such as: negative staircase, reverse staircase, descending staircase, rest potentiation, or "Woodworth phenomenon" (9, 10).

In the present study we have explored the influence of the rate of contraction (electrically induced, following two different experimental designs) on the isometric developed tension amplitude (IDTA) of left auricles isolated from rats and guinea pigs. Furthermore, in an attempt to verify whether the frequency–force relationship of guinea pig and rat atria is influenced by tissue neurotransmitters, the effects of cholinergic and adrenergic receptor antagonists on this phenomenon were also studied.

Materials and Methods. Male albino rats and male guinea pigs, weighing between 200–250 g and between 400–500 g, respectively, were used. The animals were sacrificed by decapitation, and immediately following killing the entire heart was quickly excised and dropped into a petri dish filled with a modified Krebs–Ringer–Bicarbonate solution (KRB), kept at room temperature and composed as follows (m*M*): Na^+, 145; K^+, 6.00; Ca^{2+}, 1.75; Mg^{2+}, 1.33; Cl^-, 126; HCO_3^-, 25.30; SO_4^{2-}, 1.33; and PO_4^{2-}, 1.20. The substrate for this solution was glucose at 5.5 m*M*. It was gassed with 95% O_2 and 5% CO_2. The auricles were separated from the ventricles and dissected free from all obvious extraneous tissue. The right atrium was discarded whereas the left one was transferred to a glass organ bath containing 20 ml of KRB–glucose solution and examined for contractile activity. The tension developed by atria (peak tension) was recorded in a similar way to that previously reported (12, 13). Briefly, the left atrium was attached to a glass holder, immersed into the tissue bath solution, and subjected to a resting tension of 750 mg by means of a micrometric device. The tension developed

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by the preparations was recorded with a strain gauge, coupled to an ink-writing oscillograph. With the experimental setup employed in this study, atrial preparations contracted under almost nearly isometric conditions. The nutrient KRB–glucose solution of the organ bath was kept at a constant temperature of 30° and at pH 7.4 and gassed through a fritted glass cylinder with a mixture of 95% O_2–5% CO_2 throughout the experiments. An equilibration period of 60 min was allowed before records of IDTA (measured in mg) were taken. Atrial preparations were driven with slightly suprathreshold (±10%) electrical square waves of 0.5-msec duration, delivered via two punctate platinum electrodes. The rates of stimulation were 25, 50, 100, or 200 pulses/min. Two different procedures of stimulation, which will be identified as type A and type B, were employed. One group of atrial preparations was electrically driven following procedure type A—the different increasing frequencies of stimulation (25, 50, 100, and 200 pulses/min) were applied stepwise to each atrium. Following a 5–10-min period of stimulation at each one of these frequencies, records of IDTA were obtained. Another group of atrial preparations was driven following procedure type B—each atrium was electrically stimulated during a period of 5–10 min at only one of the previously mentioned frequencies. Immediately after each period IDTA readings were taken.

The influence of atropine sulfate (Sigma Chemical Co.), H 56/28 (1-(-ally-phenoxy)-3-isopropylamino-2-propanol) (Aptin, Astra), or phentolamine mesylate (Regitine, Ciba) at 4, 0.2, and 5 μg/ml (final salt concentration in the tissue bath medium), respectively, were explored. Freshly prepared solutions of these agents were added to the suspending medium in volumes never exceeding 0.2 ml.

In the experiments designed to test the effects of the previously mentioned drugs, atrial preparations were exposed to their presence during 15–20 min prior to the initiation of the electrical stimulation. Results (IDTA, expressed in mg) under the different experimental conditions were compared as means by the Student's *t* test, using the tables given by Fisher and Yates (14). Differences between means were considered significant if $P = 0.05$ or less.

Results. Effect of the frequency of stimulation on the isometric developed tension amplitude of isolated left rat atrium. Figure 1 depicts the absolute levels of IDTA of isolated left rat auricles driven at various frequencies of stimulation applied to the tissue with procedure type A or type B. It can be seen that with procedure type A (Fig. 1, panel I), as the rate of stimulation is increased stepwise (from 25 to 200/min), the IDTA suffered a distinct stepwise decrement.

On the other hand, with procedure type B (Fig. 1, panel II), the IDTA amplitude was comparable at all the frequencies studied. Therefore, the negative staircase phenomenon of isolated left rat atrium is only evident in preparations stepwise stimulated at increasing frequencies, whereas no changes in the IDTA associated with the rate of stimulation were observed in those

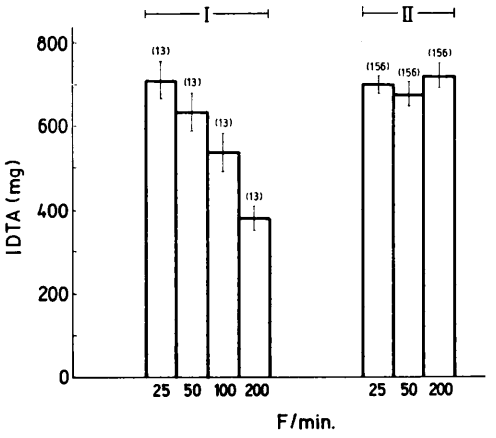

FIG. 1. Effect of the frequency of stimulation on the isometric developed tension amplitude (IDTA) of isolated left rat atria. (I) Left atrial preparations electrically driven following procedure type A (see Methods). (II) Left atrial preparations electrically driven following procedure type B (see Methods). Each column represents the mean absolute value of IDTA (in mg) of auricles electrically stimulated at different frequencies per minute (F/min). Numbers in the parentheses refer to the number of preparations. Vertical bars indicate the SEM.

driven at only one of the same frequencies. The absence of descending staircase with procedure type B results from a greater contractile tension at the highest frequency of stimulation (200/min).

Effect of the frequency of stimulation on the isometric developed tension amplitude of isolated left guinea pig atrium. The stepwise increment of the rate of stimulation with procedure type A (Fig. 2, panel I) resulted in a stepwise augmentation of IDTA of guinea pig atria in a range between 25 and 100 pulses/min. A further increment of the stimulatory frequency (up to 200/min) failed to produce higher levels of IDTA. A similar response was observed following type B stimulation (Fig. 2, panel II). Therefore, guinea pig atria respond with ascending staircase regardless of the stimulating procedure, this being in contrast to the behavior of rat atria. However, it should be noticed that with procedure type B the increment of IDTA within the range of 25–100 pulses/min was significantly smaller than that observed following procedure type A.

Influence of atropine on the frequency–force relationship of isolated left rat atrium. The effect of atropine, a well-known cholinergic blocking agent (15), upon the influence of the frequency of stimulation on the IDTA of isolated rat auricles, driven electrically with procedure type A or type B, was explored. As shown by Fig. 3, the IDTA of atria pretreated with atropine and stimulated with procedure type A was comparable at all the rates studied (Fig. 3, panel I), i.e., atropine abolished the negative staircase observed in untreated controls (Fig. 1, panel I). This was attained mainly at the expense of a distinct increment in the IDTA at the higher frequencies (50, 100, and 200/min); but an augmentation, although smaller, was also seen at the lowest rate (25/min).

On the other hand, the absence of staircase phenomenon (ascending or descending) exhibited by nonatropinized control atria driven with procedure type B (Fig. 1, panel II) remained unaltered in the presence of atropine (Fig. 3, panel II). With this type of stimulation, the IDTA after atropine was not only similar at all the frequencies studied (Fig. 3, panel II) but also comparable to that of nonatropinized controls (Fig. 1, panel II).

Influence of H 56/28 and/or phentolamine on the frequency–force relationship

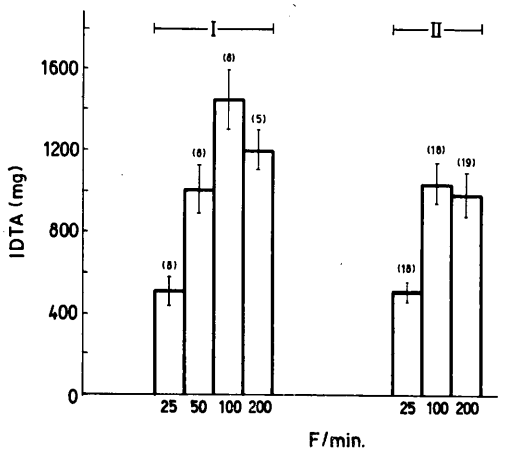

FIG. 2. Effect of the frequency of stimulation on the isometric developed tension amplitude (IDTA) of isolated left guinea pig atria. Conditions and details as described for Fig. 1.

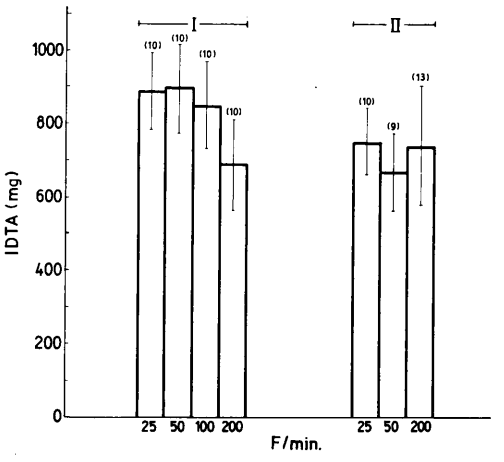

FIG. 3. Influence of atropine on the frequency–force relationship of isolated left rat atria. (I) Atropinized left atrial preparations (atropine at 4 μg/ml final concentration in the tissue bath solution) electrically driven following procedure type A (see Methods). (II) Atropinized left atrial preparations (same concentration) electrically driven following procedure type B (see Methods). Other conditions and details as described for Fig. 1.

of isolated left guinea pig atrium. The effect of alpha and/or beta adrenergic receptor blockers upon the frequency–force relationship of isolated guinea pig auricles driven electrically with procedure type A, was explored. Figure 4 shows that after H 56/28, a beta adrenergic receptor antagonist (16), a positive staircase phenomenon, similar to that of untreated controls, still can be produced (Fig. 4, panel I). However, a comparsion between atrial contractile response with and without H 56/28 indicates that in its presence, the IDTA of preparations driven with 25, 50, or 100 pulses/min, but not those stimulated at 200/min, was significantly smaller than that of untreated controls (Fig. 2, panel I).

On the other hand, a preincubation with phentolamine, a well-known alpha adrenergic receptor antagonist (17), failed to abolish completely the ascending staircase phenomenon of guinea pig atria, at least within a certain range of frequencies (Fig. 4, panel II). It should be noticed that in the presence of phentolamine, a distinct increment of IDTA at the lower frequencies (25 and 50/min), as compared to those of untreated controls (Fig 2, panel I), was also evident.

Figure 4 also illustrates the effect of the presence of H 56/28 plus phentolamine on the frequency–force relationship of isolated guinea pig atria. As can be seen (Fig. 4, panel III), these agents abolished completely the positive staircase phenomenon of isolated guinea pig auricles, the IDTA becoming similar at all the frequencies studied. A comparison with the situation in untreated controls (Fig. 2, panel I) indicates that the disappearance of the positive staircase following H 56/28 plus phentolamine occurred at the expense of a significant diminution of the IDTA at the higher frequencies (100 and 200/min), this being accompanied by a small increment of contractile tension at the lowest frequency (25/min) and by no significant changes at 50/min.

Discussion. This study provides evidence that auricles isolated from rats and guinea

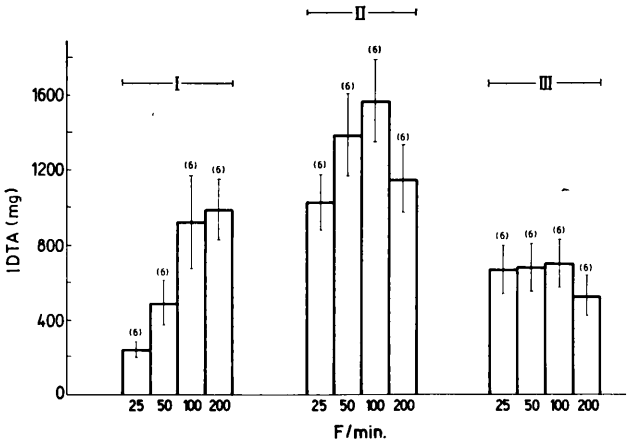

FIG. 4. Influence of H 56/28 and/or phentolamine on the frequency–force relationship of isolated left guinea pig atria. (I) Left atrial preparations electrically driven following procedure type A (see Methods). Experiments were performed in the presence of H 56/28 (0.2 μg/ml final concentration in the tissue bath solution). (II) Left atrial preparations electrically driven following procedure type A (see Methods). Experiments were performed in the presence of phentolamine (5 μg/ml final concentration in the tissue bath solution). (III) Left atrial preparations electrically driven following procedure type A (see Methods). Experiments were performed in the presence of H 56/28 plus phentolamine (0.2 and 5 μg/ml, final concentration in the tissue bath solution, repspectively). Other conditions and details as described for Fig. 1.

pigs have a different type of frequency–force relationship when electrically stimulated with the same procedure and within an identical range of frequencies. Indeed, when increasingly stepwise driving stimuli were applied to each preparation (i.e., procedure type A), a negative staircase was observed in isolated left rat atria but a positive one in isolated guinea pig atria. These findings are in agreement with other reports in rat (2–4, 6) and guinea pig (1, 4–6, 18) auricles.

Despite the numerous studies on this subject (19), the intimate mechanism(s) regarding the frequency–force relationship of heart preparations has not hitherto been fully clarified. The participation of changes in Ca^{2+} ion fluxes, in the total end diastolic $[Ca^{2+}]$, or in the intracellular $[K^+]$ of the muscle (9, 10, 18, 19) has been proposed. Also, the ascending staircase has been considered associated with a catecholamine release from tissue storage sites (11, 20). This is in keeping with observations that electrical stimulation is able to release neurotransmitters from heart muscle (21) and that reserpinization (22), blockade of adrenergic beta receptors (23), or denervation (24) can alter the positive inotropic effect of frequency. However, some authors have cast doubts about the role of catecholamines in the frequency–force relationship of heart muscle (10, 22). In the present experiments, the positive staircase of isolated left guinea pig atria was not abolished when alpha or beta adrenergic receptor antagonists were present separately, but the phenomenon disappeared after the combined presence of both agents. Therefore, it is reasonable to consider that an adrenergic mechanism is somehow involved in the frequency–force relationship of guinea pig atria. The possibility exists that high frequencies of stimulation increase catecholamine release (presumably norepinephrine) from tissue storage sites or, alternatively, that high rates of stimulation decrease neuronal or extraneuronal catecholamine reuptake. Also, differences in the neurotransmitter–receptor affinity, in the intrinisic responsiveness of effector cells, or even in the extraneuronal metabolic breakdown of released catecholamines, at the various rates of stimulation, cannot be excluded as a mechanism underlying the frequency–force relationship. Although less feasible, a nonspecific cardic depressant action exerted at high frequencies by the combined treatment of phentolamine and H 56/28 is to be kept in mind. The persistence of a positive inotropic effect of frequency after an adrenergic beta receptor antagonist, such as H 56/28, could be explained assuming that the response is mediated by released endogenous norepinephrine acting upon free alpha adrenergic receptors. Indeed, it has been recently reported that cardiac positive inotropic effects are exerted via alpha adrenergic receptors (25, 26). On the other hand, when an alpha adrenergic receptor blocker, such as phentolamine, is present, the ascending staircase might result from the interaction of released endogenous norepinephrine with free beta adrenergic receptors.

A different situation was observed in isolated left rat auricles, i.e., the stepwise increment of the stimulating frequency results in a stepwise negative staircase. It has been suggested that in heart preparations the negative and positive inotropic influence of frequency might be associated with the release from storage sites of acetylcholine or norepinephrine respectively (27, 24). This notion is supported by the finding that cardiac electrical stimulation can release both cholinergic and adrenergic autonomic mediators (28). The present experiments show that the negative staircase of isolated left rat atrium is abolished by atropine. Therefore, it would appear that in rat auricles the stepwise increment of frequency, attained by procedure type A, stimulates a cholinergic mechanism which depresses the IDTA. It is interesting that isolated rat atria but not guinea pig atria react with absence of staircase when stimulated with procedure type B. Apparently, this pattern of stimulation failed to trigger the previously proposed cholinergic mechanism of rat atria and thus it is not influenced by atropine. Although the experimental results do not give a final

explanation about the intimate mechanism(s) underlying the frequency–force relationship in different heart muscle preparations, they provide evidence suggesting a role for endogenous neurotransmitters. A cholinergic factor might determine the negative inotropic influence of frequency upon isolated rat atrium, whereas an adrenergic one could be associated with the positive inotropic influence of frequency upon guinea pig auricles. Finally, it must be noticed that, at least in isolation rat atrium, the experimental design by which the tissue is electrically stimulated seems to have paramount importance in the appearance of the staircase phenomenon.

Summary. The influence of several rates of contractions (electrically induced by means of two different experimental designs, named as type A and type B) on the isometric developed tension amplitude (IDTA) of left auricles isolated from rats and from guinea pigs were studied. Furthermore, the frequency–force relationship of comparable preparations in the presence of cholinergic or adrenergic alpha or beta receptor antagonists (atropine, H 56/28, or phentolamine) was also explored. Procedure type A stimulation consisted of different rates of stimulation (25, 50, 100, and 200/min) applied stepwise to each atrium, whereas with procedure type B, each atrium was stimulated at only one of the abovementioned frequencies. The IDTA of rat atria stimulated by procedure type A decreased stepwise with the increment of frequency [negative or descending staircase (N or DS)], whereas following procedure type B it was comparable at all the frequencies studied. On the contrary, the IDTA of guinea pig atria augmented stepwise with the increments in the stimulatory rate [positive or ascending staircase (P or AS)] either following procedure type A or type B. Atropine abolished the N or DS of isolated rat atria. In the presence of H 56/28 or phentolamine, a P or AS in guinea pig auricles was still observable; however, after a combined treatment with these two antagonists of beta and alpha adrenergic receptors, a complete blockade of the P or AS was obtained. It would appear that a cholinergic mechanism is involved in the N or DS of isolated rat atria, whereas an adrenergic one could be associated with the P or AS of guinea pig auricles. Furthermore, the appearance of the N or DS in isolated rat atria depends on the procedure of stimulation applied to the tissue.

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