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A Positive Influence of Frequency on the Inotropic Response of Isolated Rat Atria to Certain Adrenergic Amines. Effects of Cocaine and U-0521 (3′, 4′-Dihydroxy-2-methyl Propiophenone)[1] (39927)

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Introduction. We have previously demonstrated that the frequency-force relationship of isolated left rat atrium varied with the stimulating procedure (1). Indeed, the negative staircase observed when every atrium is driven with electrical impulses of stepwise increasing frequency (25, 50, 100, and 200 per min) failed to occur if different preparations were stimulated only at each one of all the indicated rates (1). In another words this procedure abolishes the negative influence of frequency over the contractile tension of rat atria.

In view of these findings it was decided to explore *in vitro* the effects of certain catecholamines, phenylephrine, and calcium chloride upon the contractile tension of the left rat atrium driven electrically at several frequencies with a method by which the isometric tension developed is not influenced by the stimilating rate (1). The modifications imposed by the frequency of contractions over the inotropic effect of catecholamines before and after the inhibition of two important inactivating processes of these amines, namely, the presynaptic neuronal uptake and metabolic breakdown by catechol-*O*-methyl transferase (COMT), were also examined.

Materials and methods. Male albino rats, of the Wistar strain, weighing between 200 and 250 g, were used. The animals were sacrificed by guillotine decapitation and, immediately following killing, the entire heart was quickly excised and dropped into a petri dish filled with a modified Krebs-Ringer Bicarbonate (KRB) solution, kept at room temperature, gassed with a mixture of 95% O_2–5% CO_2 composed as previously reported (1). The auricles were then separated from the ventricles and dissected free from all obvious extraneous tissue. The left atrium was transferred to a double-wall organ bath containing 20 ml of KRB solution with 5.5 m*M* glucose as the substrate and examined for contractile activity. The tension developed by atrial contractions (peak tension) was recorded as reported elsewhere (1-3). Briefly, the left atrium, anchored by one pole to a glass holder and having the opposite pole tied with a silk thread, was immersed into the tissue-bath solution, connected by the thread to the sensitive head of a strain gauge (Statham UC3, Gold Cell) and subjected to a constant resting tension of 750 mg by means of a micrometer attached to the transducer. The output of the transducer was amplified with an electronic device (San Ei, Biophysiograph-180) and recorded by means of ink-writing oscillograph (Rectigraph 8-S). With the described experimental set up, atrial preparations contracted under almost isometric conditions.

In order to avoid artifacts evoked by dissection trauma, an equilibration period of 60 min was allowed before control records of the isometric developed tension (IDT, measured in milligrams) were taken.

Atrial preparations were driven with slightly suprathreshold (+10%) electrical square pulses of 0.5-msec duration, delivered by a conventional stimulator (SAEN, EB-100) and conveyed to the tissue via two platinum electrodes included in the glass

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holder. The rates of stimulation used were 25, 50, 100, and 200 pulses per min. Four different groups of auricles were electrically driven, each at only one of these frequencies. With such a method the tensions developed by the preparations of all the groups were similar, i.e., not influenced by the stimulating rate. This finding is in keeping with the observation made in a previous study (1).

Mean tension values (in milligrams) recorded at 10 min, immediately after equilibrium (zero time), and prior to the addition of any drug were considered as the absolute initial control magnitudes. The effects of different compounds were then explored during a variable period of time and the magnitudes of IDT obtained under their influence were compared with those of initial controls (considered as 100%) and expressed as percentage changes. Some atrial preparations, driven at the various frequencies, were exposed to norepinephrine, isoproterenol, phenylephrine, or calcium chloride. In other cases, cocaine or U-0521 was incorporated to the bath solution 20 min before the addition of norepinephrine or isoproterenol, in order to ascertain whether or not they were capable to alter the inotropic response to these sympathetic agonists.

Freshly prepared solutions of the following agents, in volumes never exceeding 0.2 ml, were used: L-norepinephrine bitartrate (Sigma Chemical Co.) at 3.3×10^{-7} *M*; DL-isoproterenol-HCl (Sigma Chemical Co.) at 8.0×10^{-8} *M*; L-phenylephrine-HCl (Sigma Chemical Co.) at 1.2×10^{-7} *M*; cocaine-HCl (Souberan Chobet) at 1.0×10^{-5} *M*; calcium chloride·$2H_2O$ (Mallinckrodt) at 5.0×10^{-3} *M*; and U-0521 (3′, 4′-dihydroxy-2-methyl propiophenone)[4] at 3.0×10^{-4} *M*. All these concentrations represent the final ones in the suspending medium. The doses of positive inotropic agents were chosen on the basis of: (a) production of a measurable contractile effect at all frequencies; (b) capacity to elicit at one beating frequency a contractile enhancement of about the same magnitude (for this purpose pilot experiments proved the usefulness of tests at a frequency of 25 per min); and (c) absence as much as possible of influences which may complicate the interpretation of results.

The requirement indicated in (b) was considered of utmost importance in order to have an identical rate similar control values to be used as a reference point for valid comparisons with results at other frequencies. The condition stated in (c) was particularly important for the case of phenylephrine, which at a higher concentration can alter neuronal uptake. The doses of cocaine and U-0521 are those known to modify effectively neuronal uptake and adrenergic amine metabolism by COMT, respectively.

Results. Inotropic effect of norepinephrine upon isolated left rat atrium driven at different frequencies. Figure 1 depicts the effect of norepinephrine on the isometric developed tension of atrial preparations stimulated with 25, 50, 100, and 200 pulses per min. As can be seen, the positive inotropic action of the sympathetic agonist varied with the beating frequency. There was a significant difference between values obtained at 25 or 50 per min and those at 100 or 200 per min.

It is important to remark that the percentage changes of atrial isometric developed tension, elicited by norepinephrine, are not

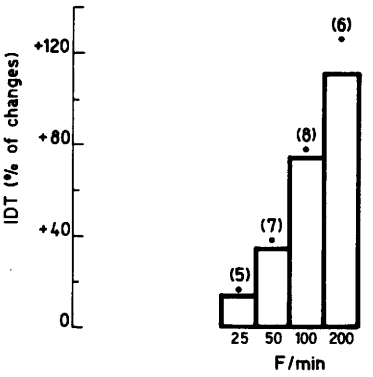

FIG. 1. Effect of norepinephrine upon the isometric developed tension (IDT) of isolated left rat atrium. Each column represents the mean value expressed as percentage change against the initial control tension developed by auricles electrically stimulated at different frequencies per min (F/min). Numbers in parentheses refer to the number of preparations. Points indicate the SEM.

[4] Courtesy of Dr. J. E. Pike, The Upjohn Co., Kalamazoo, Mich.

determined by the amplitude of the contractions before adding the amine. Indeed, the absolute control magnitude of atrial isometric tension before norepinephrine was similar at all frequencies tested (Table I).

Inotropic effect of isoproterenol, phenylephrine, and $CaCl_2$ upon isolated left rat atrium driven at different frequencies. Figure 2 illustrates comparatively the positive inotropic effect of isoproterenol, phenylephrine, and $CaCl_2$, over atrial preparations stimulated with 25, 50, and 200 pulses per min. As can be seen (Fig. 2A) the influence of isoproterenol upon isometric developed tension follows a profile similar to that of norepinephrine (Fig. 1). Indeed, it varied with the frequency, being significantly smaller at the lowest rate (25 per min) and becoming progressively greater at the higher frequencies. On the other hand, phenylephrine (Fig. 2B) and $CaCl_2$ (Fig. 2C) similarly enhanced atrial contractions at all the tested driving rates.

Table I shows that the absolute control magnitude of isometric developed tension, prior to the addition of each drug, was similar at 25, 50, and 200 beats per min.

Influences of cocaine and U-0521 on the positive inotropic effect of norepinephrine over isolated left rat atrium driven at different frequencies. Figure 3 shows the positive inotropic effect of norepinephrine (Fig. 3A) upon atrial preparations stimulated with 25, 50, and 200 pulses per min as well as the modifications induced over this action by the presence of cocaine (Fig. 3B); a blocker of presynaptic neuronal uptake of catecholamines, or by U-0521 (Fig. 3C), an inhibitor of their breakdown via COMT. As can be observed, the incubation with cocaine or U-0521 significantly enhanced the stimulating effect of norepinephrine on auricles beating at a rate of 25 and 50 per min; but

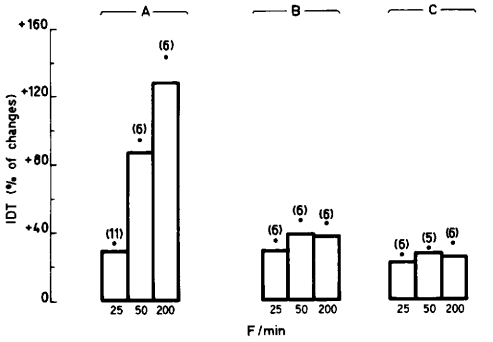

FIG. 2. Effect of isoproterenol (A), phenylephrine (B), and $CaCl_2$ (C) upon the isometric developed tension (IDT) of isolated left rat atrium driven at different frequencies. Other conditions and details one as decribed for Fig. 1.

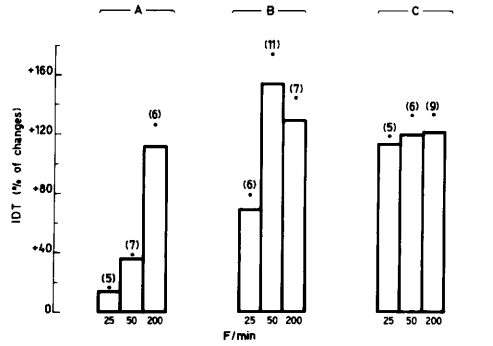

FIG. 3. Influence of cocaine and U-0521 on the positive inotropic effect of norepinephrine over isolated left rat atrium driven at different frequencies. (A) Effect of norepinephrine alone; (B) effect of norepinephrine after cocaine; (C) effect of norepinephrine after U-0521. Other conditions and details are as described for Fig. 1.

TABLE I. ABSOLUTE MAGNITUDE OF THE ISOMETRIC DEVELOPED TENSION OF RAT AURICLES DRIVEN AT DIFFERENT FREQUENCIES. EFFECTS OF COCAINE AND U-0521.

Beating frequency	Isometric developed tension (mg)[a]		
	Controls without additions[b]	Cocaine[c] (1.0×10^{-5} *M*)	U-0521[c] (3.0×10^{-4} *M*)
25 per min	656.1 ± 40.7 (28)	630.0 ± 50.1 (28)	646.4 ± 58.1 (11)
50 per min	602.2 ± 40.0 (24)	573.8 ± 38.4 (16)	650.0 ± 54.3 (13)
200 per min	631.3 ± 38.2 (24)	605.8 ± 53.4 (12)	629.3 ± 52.7 (14)

[a] Mean magnitudes ± SEM. Numbers in parentheses indicate the number of preparations.
[b] Initial values recorded at 10 min following equilibrium (see Materials and Methods).
[c] Values taken at 20 min after drug addition.

failed to induce alterations over those driven at 200 per min (see Figs. 3B and C).

As in the previous cases, before the addition of norepinephrine the absolute IDT magnitudes of atria stimulated at different frequencies and exposed to cocaine or U-0521 were comparable (Table I).

Influences of cocaine and U-0521 on the positive inotropic effect of isoproterenol upon isolated left rat atrium driven at different frequencies. Figure 4 depicts the positive inotropic effect of isoproterenol over atrial preparations stimulated with 25, 50, and 200 pulses per min (Fig. 4A) as well as the changes imposed by an incubation with cocaine (Fig. 4B) or with U-0521 (Fig. 4C). The presence of cocaine did not modify the action of isoproterenol at any of the frequencies explored (see Fig. 4B), whereas U-0521 enhanced the positive inotropism produced by the agonist at 25 or 50 beats per min but not at 200 per min (see Fig. 4C).

Discussion. In the present study we have shown that the magnitude of the positive inotropic effects of norepinephrine and isoproterenol over isolated left rat auricles varied with the frequency of contractions. They were smaller at lower rates and greater at higher rates. A negative influence of the frequency upon the epinephrine-induced positive inotropism has been found in guinea pig atria (14). However, inasmuch as the study was performed over guinea pig auricles, a tissue that reacts to the increment of frequency with a positive staircase (1), the diminished effect of the agonist at a fast rate could be due to the limitation imposed by the magnitude of contractile tension prior to addition.

Based on experiments on the frequency-force relationship of cat, guinea pig, and rabbit myocardium, it has been postulated (5, 6) that the degree of the contractile action of norepinephrine and of certain other stimulating factors is always determined by the amplitude of tension (associated in turn to the frequency) existing before introducing, the inotropic interventions. Nevertheless, the results of the present study document some limitations of this generalization. Indeed, our experimental series were performed at various frequencies but employing a procedure by which the absolute values of contractile tension before drug additions were similar. Under these conditions some agents (phenylephrine, calcium chloride) elicited a comparable positive inotropism at all frequencies, whereas others (norepinephrine, isoproterenol) produced a different intensity of contractile enhancement depending on the beating rate. Therefore, it is clear that the variable positive inotropism of the last two agonists over rat auricles driven with 25, 50, 100, and 200 pulses per min cannot be based on differences in the magnitude of tension existing before their addition.

These results prompted us to explore whether the influence of frequency over the contractile effects of norepinephrine and isoproterenol might be altered by the inhibition of some of the processes known to

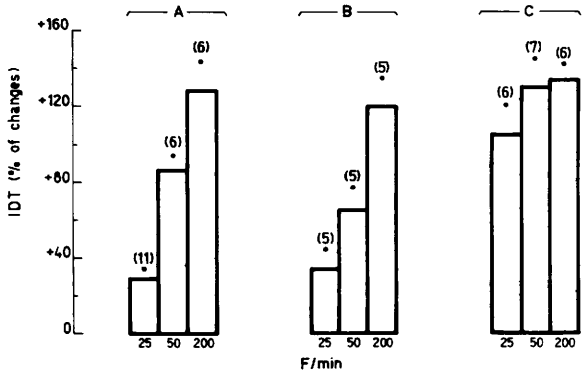

FIG. 4. Influence of cocaine and U-0521 on the positive inotropic effect of isoproterenol over isolated left rat atrium driven at different frequencies. (A) Effect of isoproterenol; (B) effect of isoproterenol after cocaine; (C) effect of isoproterenol after U-0521. Other conditions and details are as described for Fig. 1.

be involved in the inactivation of catecholamines. Cocaine, a blocker of the presynaptic neuronal uptake of some adrenergic agonists (7, 8) augmented the positive inotropic effect of norepinephrine over slowly beating atria (25 and 50 per min) but not upon the fast-contracting ones (200 per min). This appears to suggest that uptake could be more relevant at low frequencies and if so it might explain the diminished action of this agonist at reduced rates. This hypothesis is in keeping with results in rat and guinea pig atria, documenting a maximal uptake of norepinephrine and epinephrine at a low frequency (60 per min) whereas at a higher one (120 per min) uptake was found to be practically nill (4, 9, 10).

A different situation exists regarding isoproterenol. The fact that its action was not altered by cocaine, at any of the frequencies tested, could be explained considering that this agonist is not taken up by presynaptic terminals (4, 11, 13, 14).

The results reported in this study also suggest that neuronal uptake is neither the only nor the most important factor accounting for the variable inotropic influences or norepinephrine at different frequencies. Other factors involved in the termination of action of adrenergic amines, namely, metabolism by COMT, could play an important role. Indeed, in the presence of U-0521 (3.4×10^{-4} *M*), a competitive inhibitor of COMT (14), the effects of norepinephrine and isoproterenol became potentiated, although only at the lower frequencies (25 and 50 per min). It has been shown in spontaneously beating rabbit atria, that U-0521 (5.0×10^{-5} *M*) increased the positive inotropism of epinephrine, without being effective on that of norepinephrine (14). An augmented inotropic influence of isoproterenol after U-0521 has been observed in cat papillary muscle (15) as well as in rabbit atria (14), but the inhibitor failed to increase the contractile action of phenylephrine (16), a compound which is not a substrate for COMT (17). Furthermore the concentration of phenylephrine employed in the present study is unlikely to influence the process of presynaptic neuronal uptake (12, 13), and this may also contribute to an explanation of its lack of a variable inotropic effect at all the beating rates tested.

Our results provide evidence favoring the notion that in the isolated rat atria the positive influence of frequency over the inotropic effects of norepinephrine and isoproterenol could result from rate-modulated variations in the effectiveness of some amine-inactivating processes, namely, presynaptic neuronal uptake or metabolism by COMT. Under our experimental conditions, both mechanisms seem to be more important at low frequencies of contraction. However, as we have not yet studied the significance of another process also known to be able to terminate the action of sympathetic agonists, i.e., their extraneuronal uptake (18), we cannot ascertain at the moment its eventual role in the observed phenomena.

Summary. The effects of several inotropic interventions on the isometric developed tension (IDT) of isolated left rat atrium, electrically driven at several frequencies were investigated. The experimental stimulating procedure allowed the attainment at all the rates tested (25, 50, 100, and 200 per min), similar magnitudes of IDT prior to drug addition. Two types of contractile responses to the inotropic agents at different beating frequencies were found: (1) Norepinephrine and isoproterenol enhanced the IDT less at the slower rates (25 and 50 per min) than at the highest rate (200 per min); (2) phenylephrine and $CaCl_2$ similarly augmented atrial IDT at all driving rates. The presence of cocaine potentiated the effect of norepinephrine at lower frequencies but not at 200 beats per min; in addition, it did not modify the inotropic influence of isoproterenol at any of the rates explored. U-0521 enhanced the positive inotropism of both norepinephrine and isoproterenol only at low stimulating frequencies. The results suggest that: (a) The dissimilar effects of norepinephrine and isoproterenol at low and high rates could be associated with a different importance of the inactivating processes of these amines at the various frequencies, namely, presynaptic neuronal uptake and metabolization via COMT. Both mechanisms appear to be more important

at lower rates and this could be the reason underlying a diminished influence of norepinephrine or isoproterenol over slowly beating atria. (b) The positive influence of frequency over the inotropic effects of norepinephrine and isoproterenol is not due to the magnitude of atrial contractions existing before their addition.

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