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Differences between the prejunctional effects of phenylephrine and clonidine in guinea-pig isolated atria

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1 The prejunctional effects of clonidine and phenylephrine were studied in guinea-pig isolated atria by means of field stimulation of the sympathetic nerve terminals during the cardiac refractory period, in the presence of 1 μM atropine.

2 Clonidine (10–100 nM) produced a dose-dependent decrease in the stimulus-inotropic response curve; the IC_{50} for clonidine was increased about 70 times by the pretreatment of the preparations with 1 μM yohimbine. The effect of clonidine was not modified by 0.5 μM prazosin.

3 Unlike clonidine, phenylephrine (1–10 μM) induced a statistically insignificant increase in the contractile force of preparations stimulated at 4 Hz.

4 The inhibitory effect of phenylephrine (1–10 μM) was partially prevented by either 1 μM yohimbine or 0.5 μM prazosin. However, it was antagonized, to about the same degree as that observed with clonidine, by the pretreatment of the preparations with both 1 μM yohimbine and 0.5 μM prazosin.

5 The results seem to indicate that one component of the prejunctional effects of phenylephrine may be mediated by presynaptic α-adrenoceptors belonging to the α_1-subtype.

Introduction

Following the development of the hypothesis that prejunctional α-adrenoceptors are involved in the regulation of noradrenaline release (Langer, 1974; for reviews see Starke, 1977; Rand *et al.*, 1980), many attempts have been made to demonstrate the differences between prejunctional and postjunctional α-adrenoceptors. For this purpose, a classical pharmacological approach has been used, namely the detection of differences between the relative potencies of α-adrenoceptor agonists and antagonists (Drew, 1977; Starke, 1977) in inducing prejunctional and postjunctional effects. On the basis of these pharmacological differentiation studies, adrenoceptors have been classified into two subtypes termed α_1- and α_2-adrenoceptors (Berthelson & Pettinger, 1977). While prejunctional adrenoceptors belong to the α_2-subtype, recent evidence suggests that postjunctional receptors are not a homogeneous population and include both the α_1- and α_2-subtypes (Wikberg, 1979; DeMey & Vanhoutte, 1981; for review see McGrath, 1982).

According to recent reviews (Langer & Shepperson, 1982), phenylephrine is considered a relatively selective agonist of α_1-adrenoceptors, although previous studies have shown that, at concentrations comparable to those active on cardiac postjunctional α-adrenoceptors (Ledda *et al.*, 1975), it decreases the noradrenaline overflow caused by nerve stimulation in the rabbit isolated heart (Starke, 1972; 1973). Since the above mentioned observation was obtained in preparations pretreated with cocaine, in the present study we have attempted to re-examine the prejunctional effects of phenylephrine in untreated heart preparations, in terms of both potency and specificity. For this purpose we have compared the prejunctional effect of phenylephrine with that induced by the selective α_2-adrenoceptor agonist clonidine (Starke *et al.*, 1974). In addition we have investigated the effect on the responses to phenylephrine and clonidine, of yohimbine and prazosin, which are considered relatively selective antagonists for α_2-(Starke *et al.*, 1975) and α_1- (Cambridge *et al.*, 1977) adrenoceptors respectively.

Methods

The experiments were carried out using field stimulation of the sympathetic nerve terminals during the cardiac refractory period, according to the method previously described by Angus & Harvey (1981), with minor modifications. Isolated atria, obtained from hearts of male guinea-pigs weighing 300–400 g, were vertically mounted in a 15 ml glass chamber, containing Tyrode solution of the following composition (mM): NaCl 115, KCl 4.7, $CaCl_2$ 3.6, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 10. The solution was aerated with a gas mixture of 97% O_2 and 3% CO_2; the pH of the bathing solution was 7.4. The temperature was kept at 30°C in order to reduce the spontaneous rate and the metabolic demand of the preparations.

The length of the preparations was adjusted by stretching to produce a resting force of 1 g; this resting force was then maintained throughout the experiment.

The preparations were stimulated at a rate (4 Hz) slightly higher than the spontaneous rate, by square wave pulses of 1 ms duration and twice the threshold voltage intensity, through two platinum punctate electrodes.

The isometric contraction was recorded by an isometric transducer and a d.c. preamplifier on a pen recorder and on a dual beam oscilloscope. A block diagram of the experimental set-up is shown in Figure 1.

After a period of equilibration of at least 60 min, field stimulation was started and trains of field pulses (50 mA, 1 ms) were applied at 2 min intervals through two parallel platinum plates which ran along the whole length of the preparations. Field pulses were delivered one per consecutive contraction; a control circuit allowed timing of the field pulses to begin 10 ms after each driving pulse, during the absolute refractory period. Stimulus-inotropic response curves were obtained by increasing the number of field pulses, with the following sequence: 2, 3, 4, 6, 8 etc., until the maximum positive inotropic response was reached. The stimulus-response curves were than determined again after 10 min exposure to phenylephrine or clonidine. Preliminary experiments were done to verify that the inotropic responses to graded field stimulation remained reproducible in a given preparation for many hours, in such a way that subsequent stimulus-response curves were nearly superimposable.

Therefore, it was usually possible to test the effect of two or three different concentrations of one agonist in the same experiment. The responses induced by field stimulation were expressed as % changes from the steady-state contraction; the maximum response of the control curve was taken as 100%. Agonist IC_{50} values were calculated in each experiment, from the regression of % inhibition of the response to a train of 8 field pulses, against the logarithms of agonist concentrations; in the text they are given as the means of the antilogarithmic values within 95% confidence limits.

All the experiments were carried out in the pres-

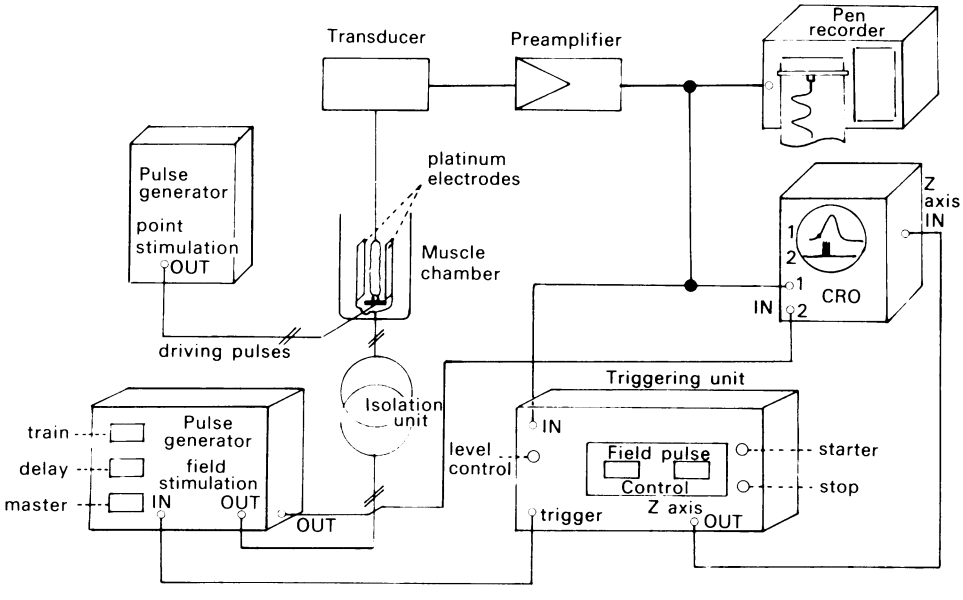

Figure 1 Block diagram of the experimental set-up.

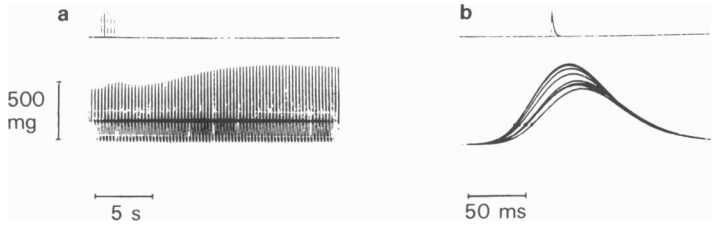

Figure 2 Effect of a train of 6 field pulses on contractile tension in guinea-pig isolated atria, recorded by an oscilloscope at two different sweep speeds. In (b) the curves have been superimposed. Stimulation rate 4 Hz; temperature 30°C.

ence of atropine (1 μM) in order to eliminate the parasympathomimetic component of the response to field stimulation.

The following substances were used: atropine sulphate (BDH), tetrodotoxin (Sankyo), reserpine hydrochloride (CIBA), 6-hydroxydopamine hydrobromide (Calbiochem), yohimbine hydrochloride (Sigma), prazosin (kindly supplied by Pfizer), phenylephrine hydrochloride (K. and K. Laboratories), clonidine hydrochloride (kindly supplied by Boehringer Ingelheim).

Results

(1) *Characteristics of the response induced by field stimulation*

Trains of field pulses applied during the atrial refractory period in the presence of 1 μM atropine induced a graded positive inotropic effect; a small response was evident after only two pulses, and a maximum effect was caused by a train of twelve field pulses. The inotropic response induced by each train lasted about 50 s and was not accompanied by any alteration of the normal rhythm or contractile wave (Figure 2).

The positive inotropic effect induced by field stimulation was strongly reduced in preparations obtained from guinea-pig pretreated with reserpine (2.5 mg kg^{-1} body wt., 24 h before the experiment) and it was completely abolished after exposure of the preparations either to 6-hydroxydopamine 2 mM for 60 min, or to tetrodotoxin 1 μM for 5 min (data not shown).

(2) *Influence of clonidine and phenylephrine on the stimulus-response curve*

Exposure of the preparations to clonidine (10–100 nM) for 10 min before stimulation, resulted in a dose-dependent depression of the stimulus response curve (Figure 3a).

The concentration of clonidine able to induce 50% inhibition (IC_{50}) of the response caused by the stimulation of sympathetic nerve terminals (calculated for the submaximal response, i.e. that induced by a train

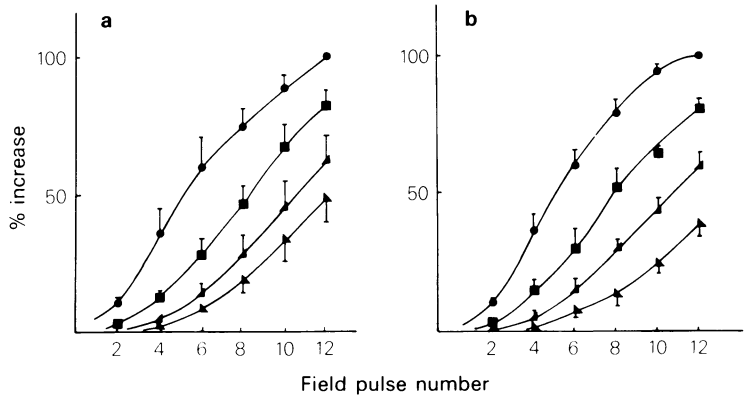

Figure 3 Effect of clonidine and phenylephrine on the sympathetic response induced by trains of field pulses in guinea-pig isolated atria stimulated at 4 Hz. (●) Control; (■) clonidine 10 nM; (◢) clonidine 30 nM; (◣) clonidine 100 nM ($n = 4$), (b) (●) Control; (■) phenylephrine 1 μM; (◢) phenylephrine 3 μM; (◣) phenylephrine 10 μM ($n = 5$). Points represent mean values and vertical bars indicate s.e.mean. Temperature 30°C.

Table 1 Concentrations of clonidine and phenylephrine able to produce a 50% inhibition (IC_{50}) of the response to a train of 8 field pulses, in untreated guinea-pig atria or in atria pretreated with prazosin and/or yohimbine

Treatment		*IC_{50} clonidine (nM)*	*95% confidence limits*
None (control)	(4)	16.9	5.2– 53.7
Yohimbine 1 μM	(4)	1202	240–5888
		IC_{50} phenylephrine (μM)	
None (control)	(5)	1.4	0.7– 2.9
Yohimbine 1 μM	(5)	14.7	6.4– 33.8
Prazosin 0.5 μM	(5)	8.4	6.9– 10.2
Yohimbine 1 μM + prazosin 0.5 μM	(4)	104.7	501.1–213.7

Numbers in parentheses represent number of experiments.

of 8 pulses) was about 17 nM (see Table 1). Unlike clonidine, phenylephrine (1–10 μM) induced a slight dose-dependent increase in contractile tension: in fact the contractile force was increased from 624.2 ± 85.3 mg to 653.3 ± 107 mg by phenylephrine 1 μM; and was increased to 654.1 ± 107.7 and to 660.4 ± 108.4 mg by phenylephrine 3 μM and 10 μM respectively (mean values ± s.e.mean of 5 experiments). However, these changes did not reach the level of statistical significance in the paired *t* test.

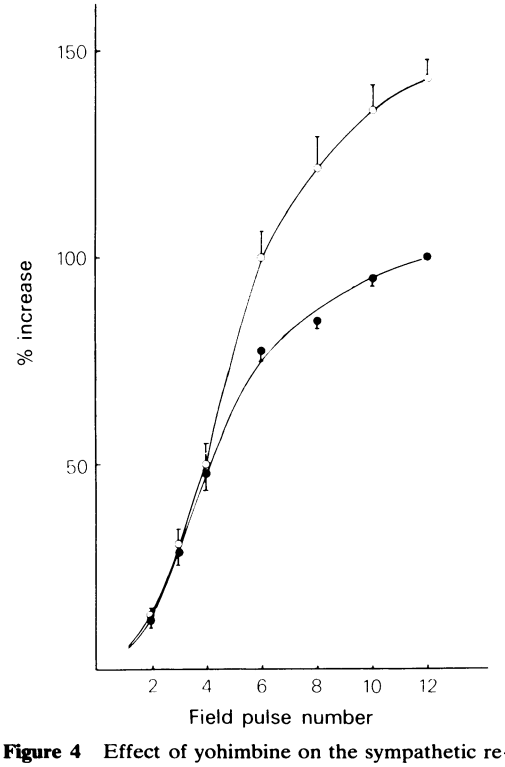

Figure 4 Effect of yohimbine on the sympathetic response induced by trains of field pulses in guinea-pig isolated atria stimulated at 4Hz. (●) Control; (○) yohimbine 1 μM ($n = 4$). Points represent mean values; vertical bars indicate s.e.mean. Temperature 30°C.

The same concentrations of the α-adrenoceptor-stimulating amine caused a dose-dependent depression of the stimulus-response curve (Figure 3b)); the calculated IC_{50} for phenylephrine was about 100 times higher than that of clonidine (Table 1). Since neither clonidine nor phenylephrine, at the same concentration used in these experiments, were able to modify the positive inotropic responses to exogenous noradrenaline, it became apparent that the site of action of both drugs was on the adrenergic nerve terminals which probably resulted in a reduction in neurotransmitter release.

(3) *Antagonism by yohimbine and prazosin of the inhibitory effects induced by clonidine and phenylephrine*

Exposure of the preparations to yohimbine 1 μM for 30 min produced a significant increase in the responses to trains of intermediate and high numbers of field pulses, while the responses induced by low numbers of pulses were completely unaffected (Figure 4). The stimulus-response curve was not significantly modified by the exposure to prazosin 0.5 μM for 30 min. Pretreatment of the preparations with yohimbine 1 μM completely prevented the inhibitory effect of 10 and 30 nM clonidine and strongly reduced that of 100 nM clonidine (Figure 5a). The mean IC_{50} of clonidine in preparations pretreated with the α_2-adrenoceptor antagonist was about 70 times higher than that in the untreated preparations (Table 1).

The effect of clonidine was completely unaffected by the pretreatment of the preparations with prazosin 0.5 μM. On the other hand, the inhibitory effect of phenylephrine 1–10 μM was only partially antagonized by yohimbine 1 μM (Figure 5b). In fact the IC_{50} for phenylephrine was increased only about 10 times in the presence of the α_2-adrenoceptor antagonist (Table 1). The phenylephrine response was also antagonized by prazosin 0.5 μM, the IC_{50} value for the agonist being increased about 6 times by the antagonist (Table 1). However, the inhibitory effect of phenylephrine was prevented to about the same de-

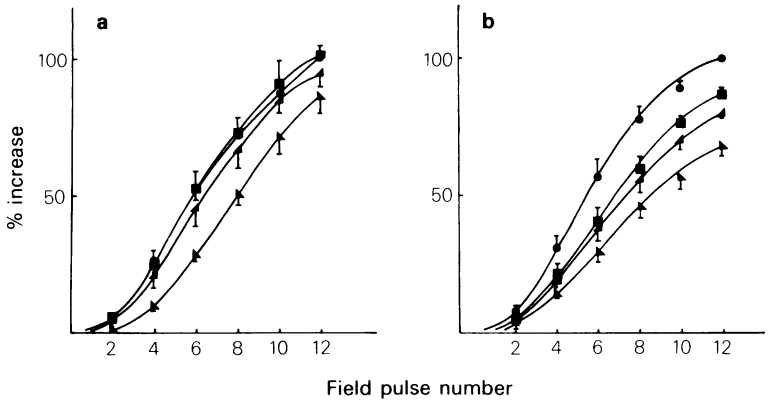

Figure 5 Antagonism by yohimbine of the inhibitory effect of clonidine and phenylephrine on the sympathetic response induced by trains of field pulses in guinea-pig isolated atria stimulated at 4 Hz. (a) (●) Control in the presence of 1 μM yohimbine; (■) clonidine 10 nM; (◢) clonidine 30 nM; (◣) clonidine 100 nM ($n = 4$). (b) (●) Control in the presence of 1 μM yohimbine; (■) phenylephrine 1 μM; (◢) phenylephrine 3 μM; (◣) phenylephrine 10 μM ($n = 4$). Temperature 30°C.

gree as that observed with clonidine, by the pretreatment of the preparations with both yohimbine 1 μM and prazosin 0.5 μM (Figure 6; Table 1).

Discussion

Most of the information about presynaptic receptors has been obtained from isolated tissue experiments in which the organ response has been used to indicate the synaptic concentration of the neurotransmitter (Doxey & Roach, 1980). Among these techniques, the method of applying electrical field pulses during the atrial refractory period, in the presence of atropine, has been shown to be suitable for investigating drug effects on cardiac sympathetic nerve terminals (Angus & Harvey, 1981; Lew & Angus, 1982), provided that trains of stimulation of suitable frequency and duration are applied (Story *et al.*, 1981). Nevertheless, controversies have arisen about the ability of this technique to demonstrate a potentiation of the sympathetic response of the heart by α-adrenoceptor-blocking agents (Angus & Korner, 1980). However, our observations obtained in preparations stimulated for periods ranging from 0.5 to 6 s at 4 Hz have clearly shown a potentiation of the inotropic response to field stimulation induced by yohimbine 1 μM. The potentiating effect of the α_2-adrenoceptor-blocking drug was more evident at the higher than at the lower numbers of field pulses. This finding can be considered to be consistent with the hypothesis that the α-adrenoceptor-mediated negative feed-back mechanism of neurotransmitter release control is predominantly activated by conditions leading to high synaptic concentrations of noradrenaline (Rand *et al.*, 1980; Langer, 1981).

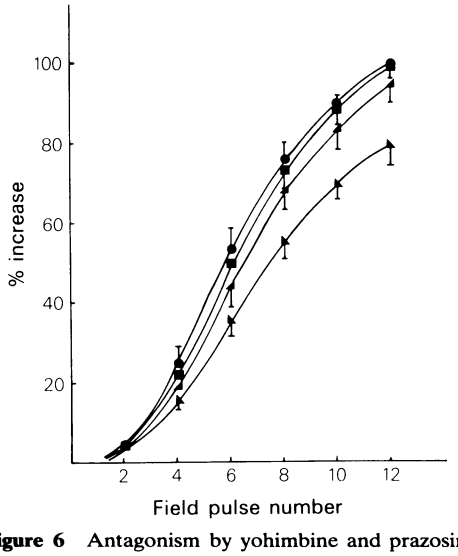

Figure 6 Antagonism by yohimbine and prazosin of the inhibitory effect of phenylephrine on the sympathetic response induced by trains of field pulses in guinea-pig isolated atria stimulated at 4 Hz. (●) Control in the presence of 1 μM yohimbine and 0.5 μM prazosin; (■) phenylephrine 1 μM; (◢) phenylephrine 3 μM; (◣) phenylephrine 10 μM ($n = 4$). Temperature 30°C.

The results obtained in the present investigation with the α_2-adrenoceptor agonist clonidine were in agreement with those found in other studies: the inhibitory effect of clonidine was dose-dependent in a range of concentrations (10–100 nM) as has been shown to occur in most peripheral preparations (Doxey & Roach, 1980). Even the observation that the inhibitory effect of clonidine was not frequency-dependent, was not surprising, since analogous re-

sults have recently been obtained by authors (Lew & Angus, 1982) using a similar experimental procedure to that employed in the present study. As expected, the effect of clonidine was considerably antagonized by yohimbine; this confirmed the well-known functional antagonism between the two drugs at presynaptic α_2-adrenoceptors in the heart (Langer & Shepperson, 1982).

As far as phenylephrine is concerned, our findings provide further support for the theory that phenylephrine is able, at concentrations comparable to those active on cardiac postjunctional α-adrenoceptors, to exert a prejunctional inhibitory effect on the sympathetic terminals of the heart (Starke, 1972; 1973). Thus, it seems conceivable that the selectivity of phenylephrine for α-adrenoceptors in the heart is not so high as in the other peripheral organs (Drew, 1977; Starke, 1977). The pattern of the inhibitory effect displayed by phenylephrine was very similar to that induced by clonidine, although the range of active concentrations was 100 times higher in the case of phenylephrine. This suggests that the inhibitory effect exerted by the two drugs on the response of the heart to sympathetic stimulation was due to a mechanism common to both. However, this interpretation was contradicted by the findings that the same concentration of yohimbine, which was able almost completely to antagonize the effect of clonidine, only partially prevented the effect of phenylephrine, and that the inhibitory effect of phenylephrine, unlike that of clonidine, was partially antagonized by prazosin. Moreover, it was only possible to counteract the effect of phenylephrine to an extent similar to that observed with clonidine, by the use of both yohimbine 1 μM and prazosin 0.5 μM. These latter observations could be explained by a trans-synaptic regulatory mechanism, since it has been previously suggested that the stimulation of postsynaptic α-adrenoceptors may lead to a decreased release of neurotransmitter (Farnebo & Malmfors, 1971).

The existence of a stimulatory effect of phenylephrine on myocardial postjunctional receptors in our experiments was indeed suggested by the appearance of a slight positive inotropic effect. The observation that this effect was not statistically significant was not surprising, since it has been previously shown that the α-adrenergic inotropic response is detectable only at relatively low stimulation rates (about 1 Hz) and that it is lost at higher rates (Ledda *et al.*, 1975; Mugelli *et al.*, 1976). Although a significantly enhanced postsynaptic response would not be detectable under our experimental conditions, one cannot obviously exclude the possibility that the occupancy of postsynaptic α_1-adrenoceptors by phenylephrine could have led to a reduced noradrenaline release, and that prazosin could have antagonized this mechanism. However, a more plausible explanation may be that both α_1- and α_2-adrenoceptors are present at the prejunctional level, and that the prazosin-sensitive component of the inhibitory effect of phenylephrine was due to the interaction of the agonist with hypothetical presynaptic α_1-adrenoceptors. In support of this hypothesis it may be borne in mind that the possibility of the presence of α_1-receptors at the prejunctional level has already been suggested (Kobinger & Pichler, 1980), and that data indicating a possible prejunctional effect of prazosin have also been recently produced (Rand *et al.*, 1982; Docherty, 1983). The findings of Docherty (1983) obtained in the pithed rat, and those of the present study in the guinea-pig isolated atria both indicate that the functional presence of prejunctional α_1-adrenoceptors would be detectable only by using agonists with a predominant affinity for α_1-adrenoceptors, since prazosin was in both studies unable to modify the presynaptic effects induced by selective α_2-adrenoceptor agonists.

Also, the observation that prazosin alone, unlike yohimbine, was unable to modify the normal stimulus-response curve, suggests that the hypothetical presynaptic α_1-adrenoceptors are not involved, to any significant extent, in the negative feedback mechanism controlling noradrenaline release from cardiac sympathetic terminals.

In conclusion, our results demonstrate that the mechanisms involved in the prejunctional effect of phenylephrine are different from those of clonidine, as they probably include both an α_1- and α_2-adrenoceptor-mediated component. Moreover, they provide further evidence for the possible presence of prejunctional α_1-adrenoceptors in cardiac tissues.

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