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CARDIAC ADRENOCEPTORS AT LOW TEMPERATURE AND THE ADRENOCEPTOR INTERCONVERSION HYPOTHESIS

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1 The hypothesis that low temperature converts inotropic β-adrenoceptors to α-adrenoceptors has been tested in isolated heart preparations of the frog and rat.

2 The results do not support the adrenoceptor interconversion hypothesis. In the frog ventricle strip lowering the temperature from 24°C to 14°C did not significantly alter the inotropic potency of the sympathomimetic drugs isoprenaline, adrenaline and phenylephrine and did not reduce the potency of the β-adrenoceptor blocking drug propranolol as an isoprenaline antagonist. In the isolated left atrium of the rat lowering the temperature from 31°C and 24°C to 17–19°C did not significantly alter the inotropic potency of isoprenaline, noradrenaline and phenylephrine, did not diminish the potency of the β-adrenoceptor blocking drug propranolol and did not increase the potency of the α-adrenoceptor blocking drug phentolamine.

3 Phenylephrine acted on α- and β-adrenoceptors in rat left atrium at 31°C and 24°C, but only on β-adrenoceptors at 17–19°C.

Introduction

Adrenoceptors can be classified into two types, α or β, according to their sensitivity to agonists and antagonists. It has been suggested that α- and β-adrenoceptors are 'conformations' of a single adrenoceptor (Kunos & Szentivanyi, 1968; Kunos, Yong & Nickerson, 1973; Kunos & Nickerson, 1976). The following observation in the isolated heart of the frog has been accepted as proof of receptor interconversion (Kunos *et al.*, 1973; Kunos & Nickerson, 1976). The α-adrenoceptor blocking drug phenoxybenzamine inhibits the inotropic effect of adrenaline at low temperature and not at higher temperature; however, the adrenaline effect is inhibited at high temperature if the heart is pretreated with phenoxybenzamine at low temperature. It is assumed that at low temperature phenoxybenzamine irreversibly blocks the adrenoceptor in 'α-conformation' and that temperature elevation cannot convert it to the 'β-conformation'.

The problem is that one needs a very high concentration of an α-adrenoceptor blocking drug to inhibit the adrenaline effect on the frog heart at low temperature, which raises the question whether the inhibition is due to adrenoceptor blockade. High concentrations of α-adrenoceptor blocking drugs have inhibitory effects on the heart which are unrelated to adrenoceptor blockade.

It has further been reported that at low temperature the β-adrenoceptorblocking drug propranolol loses 90% of its potency as an antagonist of the inotropic effect of adrenaline on the frog heart (Kunos & Nickerson, 1976). The problem is that the time of treatment with propranolol was very short, which raises the question whether the receptor blockade was complete. Propranolol needs a considerable amount of time to exert its full effect at normal temperature, and low temperature further slows the development of the blockade.

Results similar to those found in frog heart have been reported from studies in isolated heart of rat (Benfey, Kunos & Nickerson, 1974).

This paper examines the evidence for the adrenoceptor interconversion hypothesis.

The adrenoceptor is a descriptive term and its nature is unknown. A response is said to be mediated by α-adrenoceptors when the relative potency of a series of agonists shows that adrenaline or noradrenaline is highest, phenylephrine is somewhat lower, and isoprenaline is very low, and when the response is susceptible to inhibition by a relatively low concentration of an α-adrenoceptor blocking drug. A response is considered to be mediated by β-adrenoceptors when measurement of the potency of a series of agonists shows that isoprenaline is highest and phenylephrine lowest, and when the response is susceptible to inhibition by a relatively low concentration of a β-adrenoceptor blocking drug.

Using these criteria I have found no evidence that

low temperature converts inotropic β-adrenoceptors in frog heart to α-adrenoceptors (Benfey, 1975, 1976). It has also been reported that adenylate cyclase-coupled β-adrenoceptors in frog, rat and dog heart do not change with temperature (Benfey *et al.*, 1974; Caron &Lefkowitz, 1974).

Kunos *et al.* (1973), Benfey *et al.* (1974) and Kunos & Nickerson (1976) did not work under 'optimal conditions in experiments for the pharmacological characterization of adrenoceptors in isolated tissues' (Furchgott, 1972). Thus in tissues containing both receptors the potency of the β-adrenoceptor blocking drug should have been determined after blockade of α-adrenoceptors. The tissue uptake processes which affect the potency of adrenaline and noradrenaline should have been blocked. The rate of the frog isolated heart should have been kept constant by pacing when the inotropic effect was recorded, because sympathomimetic drugs can exert indirect inotropic effects through chronotropic actions.

Methods

Strips of frog ventricle were suspended in a solution of the following composition (mM): NaCl 111, $NaHCO_3$ 2.4, KCl 1.9, $CaCl_2$ 1.1, NaH_2PO_4 0.07 and disodium edetate 30 μM. Contractions were elicited with square-wave pulses (1–3 ms, voltage just above threshold) at a rate of 0.3 Hz (24°C) or 0.2 Hz (14°C) and recorded isometrically. The experiments were done between the months of September and February.

Strips of rat left atrium were suspended in a solution consisting of (mM): NaCl 114.9, $NaHCO_3$ 24.9, KCl 4.7, $CaCl_2$ 1.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10 and disodium edetate 30 μM which was equilibrated with 5% CO_2 in O_2. Contractions were elicited with square-wave pulses (0.3–1 ms, voltage just above threshold) at a rate of 1 Hz (31°C), 0.5 Hz (24°C), or 0.3 Hz (17–19°C) and recorded isometrically. The rat atrium often did not respond to the sympathomimetic drugs at 17°C; the temperature was then raised to 18 and 19°C, and even then an inotropic effect was not always obtained.

Before exposure to drugs the heart preparations were maintained at a given temperature for at least an hour. Incubation with propranolol or phentolamine was for 1 h or more. The potency of the agonists was determined from cumulative dose-effect curves, a new agonist dose being added after the previous dose had reached its full effect. The potency of the agonists is expressed as the $-\log ED_{50}$ (M), which is the –log of the molar concentration that produces a half-maximal effect. The potency of the antagonists is expressed as the dose-ratio, which is the ratio of the potency of the agonist in the presence of the antagonist and in its absence.

he drugs used included: (–)-isoprenaline bitartrate dihydrate, (–)-adrenaline bitartrate and (–)-noradrenaline bitartrate (Winthrop), (–)-phenylephrine hydrochloride (K & K Laboratories), (±)-propranolol hydrochloride (Inderal; Ayerst, McKenna & Harrison), and phentolamine methanesulphonate (Ciba).

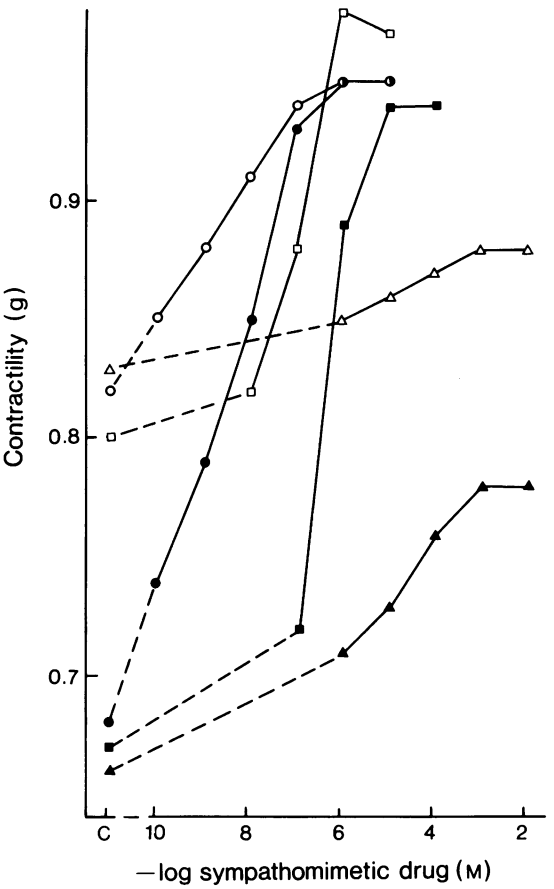

Figure 1 Effects of isoprenaline (○), isoprenaline in the presence of propranolol 0.34 μM (□), and phenylephrine (△) on contractility of frog ventricle strip at 14°C (open symbols) and 24°C (solid symbols). Means of 4–9 experiments. C: control contractility.

Results

Frog ventricle

Figure 1 shows that the contractility of the frog ventricle strips was higher at 14°C than at 24°C. Consequently, the inotropic effect of the sympathomimetic drugs was smaller at the low temperature than at the higher temperature. The

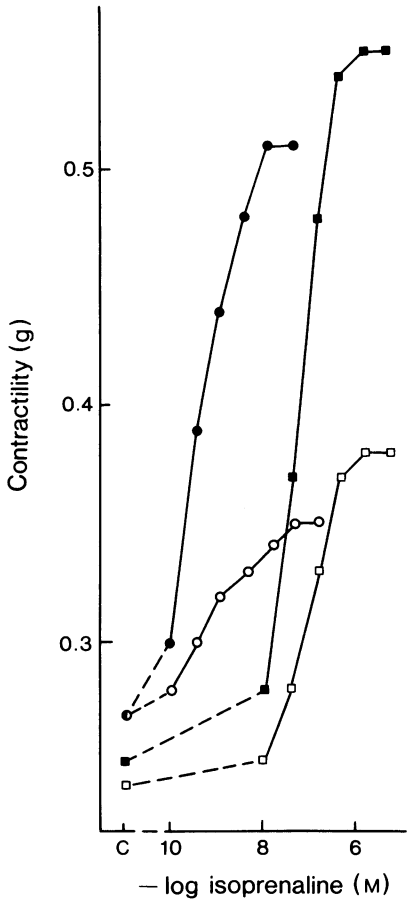

Figure 2 Effects of isoprenaline in the absence (O) and presence of propranolol 0.1 μM (□) on contractility of rat left atrium at 17–19°C (open symbols) and 31°C (solid symbols). Means of 5 experiments. C: control contractility.

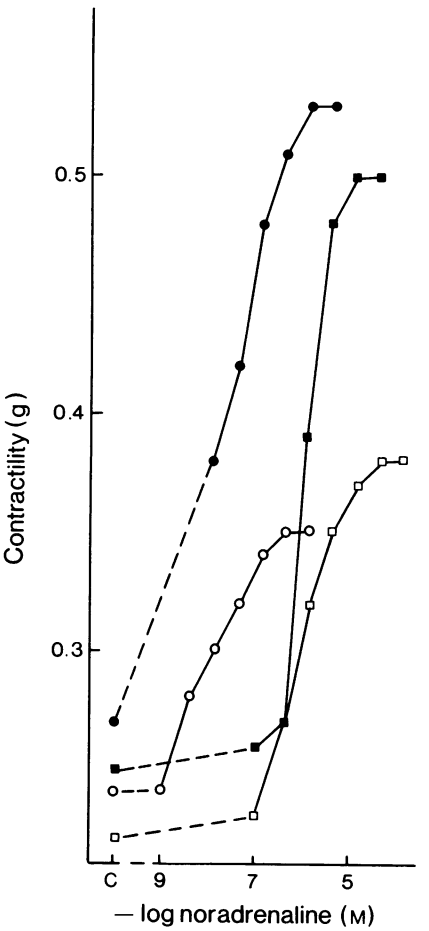

Figure 3 Effects of noradrenaline in the absence (O) and presence of propranolol 0.1 μM (□) on contractility of rat left atrium at 17–19°C (open symbols) and 31°C (solid symbols). Means of 5 experiments. C: control contractility.

inotropic effect of adrenaline was similar to that of isoprenaline, but that of phenylephrine was smaller, both at 24°C and at 14°C.

Lowering the temperature did not significantly change the potency of isoprenaline, adrenaline and phenylephrine. Isoprenaline remained the most potent, and phenylephrine the least potent drug. The potency of propranolol as an isoprenaline antagonist did not change with temperature.

Rat atrium

Lowering the temperature from 31°C to 17–19°C did not change the level of the contractility of the rat atrium but reduced the effect of the inotropic drugs (Figures 2, 3 and 4). The effect of phenylephrine was similar to that of isoprenaline and noradrenaline.

The potency of isoprenaline, noradrenaline and phenylephrine and the potency of propranolol as an antagonist of isoprenaline and noradrenaline did not significantly change when the temperature was reduced from 31°C to 24°C and 17–19°C.

Phenylephrine behaved unexpectedly. The inotropic effect of phenylephrine was inhibited by propranolol and phentolamine at 31°C and 24°C, but only by propranolol at 17–19°C.

These results suggest that in the rat atrium at 17–19°C isoprenaline, noradrenaline and phenylephrine act solely on β-adrenoceptors and that at 31°C and 24°C isoprenaline and noradrenaline act solely on β-adrenoceptors and phenylephrine acts on α- and β-adrenoceptors.

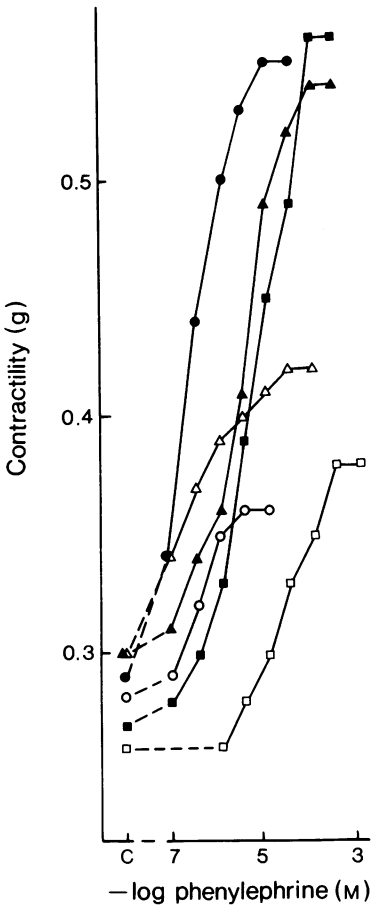

Figure 4 Effects of phenylephrine in the absence (O) and presence of propranolol 0.1 μM (□) and phentolamine 1 μM (△) on contractility of rat left atrium at 17–19°C (open symbols) and 31°C (solid symbols). Means of 5 experiments. C: control contractility.

Discussion

Amphibian hearts

There are four papers that deal with the potency of sympathomimetic drugs on the amphibian heart at low temperature, and their results are varied. For example, temperature reduction from 25°C to 12°C caused a tenfold increase in the chronotropic potency of adrenaline in the perfused toad heart (Harri, 1973) but temperature reduction from 24°C to 14°C led to a tenfold crease in the inotropic potency of adrenaline in the frog isolated heart (Kunos & Nickerson, 1976). At 12°C isoprenaline was more potent than phenylephrine in the perfused toad heart (Harri, 1973) and frog heart (Tirri, Harri & Laitinen, 1974); at 14°C isoprenaline was more potent than phenylephrine in the frog ventricle (Benfey, 1975).

There are three papers that deal with the potency of β-adrenoceptor blocking drugs on the amphibian heart at low temperature, and their results vary. For example, temperature reduction from 25°C to 12°C caused a fourfold decrease in the potency of propranolol as an isoprenaline antagonist in the perfused toad heart (Harri, 1973) and temperature reduction from 24°C to 14°C led to a slight increase in the potency of propranolol as an isoprenaline antagonist in the frog ventricle (Benfey, 1975). Temperature reduction from 25°C to 12°C caused a slight increase in the potency of propranolol as an adrenaline antagonist in the perfused toad heart (Harri, 1973) but temperature reduction from 24°C to 14°C led to a ten- to thirty-fold decrease in the potency of propranolol as an adrenaline antagonist in the isolated heart of the frog (Kunos & Nickerson, 1976).

Kunos & Nickerson (1976) treated their preparations with propranolol for only 10 min, and this does not appear to be a sufficient time to obtain a full receptor blockade, especially at low temperature. At 37°C it took 90 min to achieve a 98% equilibration with propranolol in guinea-pig isolated atria (Potter, 1967) and 75 min to obtain an 80–90% equilibration in guinea-pig isolated trachea (Furchgott, Jurkiewicz & Jurkiewicz, 1973). It is suggested that the reduction in potency of propranolol observed by Kunos & Nickerson (1976) is caused by an incomplete receptor blockade.

Turning to α-adrenoceptor blocking drugs: Nickerson & Nomaguchi (1950) reported that the chronotropic effect of adrenaline on the winter frog heart was inhibited by low concentrations of several β-haloalkylamines, e.g. dibenamine 3.4 μM; the inotropic effect was not inhibited. However, the blocking action of the β-haloalkylamines differed from their action on mammalian smooth muscle in that the blockade did not persist after the perfusion containing the active agent was replaced by fresh frog-Ringer solution.

Buckley & Jordan (1970) tested the adrenoceptor interconversion hypothesis by incubating the frog heart at 7°C with phenoxybenzamine 15 μM for 90 minutes. The chronotropic and inotropic effects of adrenaline were inhibited at 7°C, but the adrenaline antagonism disappeared when the temperature was raised to 24°C.

A persistent adrenaline antagonism was obtained by incubating the frog heart at 14°C with 7.3 μM phenoxybenzamine in four consecutive 10 min periods (Kunos *et al.*, 1973; Kunos & Nickerson, 1976). The effect was described as that of a 40 min incubation with 7.3 μM phenoxybenzamine, but it is that of a much higher concentration, or dose of the drug, because phenoxybenzamine cumulates and exerts pro-

gressively greater effects when administered repeatedly (Furchgott, 1966). Phenoxybenzamine is unstable in aqueous solution at neutral pH. Only part of the drug is available for reaction with tissues, and this part will alkylate a certain proportion of nucleophilic sites. Administration of fresh drug will continue this process.

The concentration of phentolamine used by Kunos & Nickerson (1976) to inhibit the inotropic effect of adrenaline (26.5 μM) also is much higher than that needed to block α-adrenoceptors in other tissues. The conclusion that the high concentrations of the α-adrenoceptor blocking drugs do not inhibit the adrenaline effect by adrenoceptor blockade is consistent with the observation that at low temperature phenoxybenzamine also inhibits the effect of isoprenaline (Benfey, 1975; Kunos & Nickerson, 1976), although there is evidence that isoprenaline does not act on α-adrenoceptors (Figure 1).

It is of interterest that phenoxybenzamine 2.9 μM inhibits the inotropic effect of acetylcholine on frog isolated ventricle (Benfey, 1975). Thus in the frog heart, phenoxybenzamine is a more potent antagonist of acetylcholine than of adrenaline.

Mammalian hearts

The mammalian heart posseses α-adrenoceptors, and α-adrenoceptors are present at normal temperature. Thus the inotropic effect of phenylephrine on the mammalian isolated heart is inhibited by α-adrenoceptor blocking drugs. α- And β-adrenoceptor mediated mechanisms differ; in contrast to β-adrenoceptor stimulation, α-adrenoceptor stimulation does not activate cardiac adenylate cyclase (Benfey, 1971; Osnes & Øye, 1975).

The concentrations of α-adrenoceptor blocking drugs which antagonize the inotropic effect of phenylephrine on mammalian heart are similar to those that block α-adrenoceptors in smooth muscle. The apparent dissociation constant of the receptor-inhibitor complex, K_B, for phentolamine (in the presence of propranolol 0.3 μM) was 28 nM in rabbit atrium (Benfey, 1973). This is in agreement with the pA_2 of 7.35 for phentolamine in rabbit papillary muscle (Schümann, Endoh & Wagner, 1974), and the K_B of 23 nM for phentolamine in rabbit aorta (Besse & Furchgott, 1976). Presynaptic α-adrenoceptors in guinea-pig atrium were blocked by phentolamine 0.31 μM (Langer, Adler-Graschinsky & Giorgi, 1977).

In the present study the K_B of phentolamine for phenylephrine antagonism was estimated (in the absence of propranolol) as 71 nM at 31°C and 49 nM at 24°C.

Higher concentrations of phentolamine can exert 'unspecific' effects. Thus phentolamine 3.1 μM inhibited the chronotropic effect of sympathetic nerve stimulation in guinea-pig atrium (Langer *et al.*, 1977).

Phenoxybenzamine is a highly potent α-adrenoceptor blocking drug. Phenoxybenzamine 1 nM inhibited the inotropic effect of phenylephrine on rabbit atrium (Benfey, 1973) and a 5 min incubation with phenoxybenzamine 0.3 nM inhibited the effect of noradrenaline on contraction of isolated spleen of the cat (Davidson & Innes, 1972). In the rabbit isolated aorta a 5 min exposure to phenoxybenzamine in a concentration of 29 nM or less significantly inhibited the effect of adrenaline (Nickerson & Chan, 1961). Dibenamine which is about 20 times less potent than phenoxybenzamine (Graham, 1962) had a pA_2 of 8.3 on 20 min exposure in guinea-pig vas deferens (Graham & Al Katib, 1966).

Higher concentrations of β-haloalkylamines have inhibitory effects on mammalian heart which are unrelated to adrenoceptor blockade. Thus 0.1 μM phenoxybenzamine inhibited the chronotropic and inotropic effects of acetylcholine on guinea-pig atrium (Benfey & Grillo, 1963); 0.78 μM phenoxybenzamine caused a 50% inhibition of neuronal noradrenaline uptake and 2.8 μM phenoxybenzamine caused a 50% inhibition of extraneuronal noradrenaline uptake in rat heart (Iversen, 1973); 10 μM dibenamine inhibited the chronotropic effect of isoprenaline (Krell & Patil, 1969), and 29 μM phenoxybenzamine inhibited the chronotropic effect of noradrenaline on guinea-pig atrium (Adler-Graschinsky, Langer & Rubio, 1972).

Benfey *et al.* (1974) reported that at 17°C four 10 min incubations with 7.4 μM phenoxybenzamine inhibited the inotropic effect of noradrenaline on rat atrium, but it is doubtful that the drug acted by adrenoceptor blockade in this high dose. Benfey *et al.* (1974) also found that lowering the temperature from 31°C to 17°C reduced the potency of propranolol as an antagonist of the ionotropic effect of noradrenaline on rat atrium, but the propranolol treatment was only done for 10 minutes.

Benfey *et al.* (1974) kept the driving rate at 1 Hz whereas I reduced it to 0.3 Hz at 17–19°C. Lowering the driving frequency should favour effects mediated by α-adrenoceptors. Thus lowering the driving frequency of the guinea-pig isolated ventricle from 2.5 to 1 Hz increased the inotropic effect of phenylephrine but not that of isoprenaline (Ledda, Marchetti & Mugelli, 1975); the inotropic effect of small concentrations of adrenaline on guinea-pig ventricle was inhibited by phentolamine at 1 Hz and by the β-adrenoceptor blocking drug practolol at 2.5 Hz (Mugelli, Ledda & Mantelli, 1976).

There are no other publications that deal with the potency of sympathomimetic or adrenoceptor blocking drugs on mammalian heart at temperatures below 25°C. It is of interest that lowering the temperature from 37.5°C to 17.5°C caused a more than ten-fold potentiation of the isoprenaline effect on guinea-pig isolated trachea (Foster, 1967).

Why did the high concentrations of the α-

adrenoceptor blocking drugs inhibit the sympathomimetic drug effects at low temperature and not at higher temperature? It is probably easier to inhibit the small effect at low temperature than the greater effect at higher temperature. Low temperature greatly reduces the rate of formation of the active phenoxybenzamine metabolite, the aziridinium ion (Harvey & Nickerson, 1953; Rosen & Ehrenpreis, 1972). Effects which depend on formation of active metabolite are depressed by low temperature. Thus low temperature greatly reduced the acetylcholine antagonism in frog ventricle and guinea-pig atrium and the tyramine antagonism and noradrenaline potentiation in guinea-pig atrium (Benfey, 1975). When the rate of formation of the active metabolite is reduced, more of the highly lipid-soluble unchanged drug may accumulate in tissues and interfere with tissue activity.

In conclusion, the following objects are raised against the adrenoceptor interconversion hypothesis. First, the concentration of an α-adrenoceptor blocking drug needed to inhibit the inotropic effect of adrenaline on the frog heart at low temperature is very much higher than that which blocks α-adrenoceptors in mammalian heart and smooth muscle. Therefore, it is doubtful whether the inhibition of the adrenaline effect is due to adrenoceptor blockade. Second, the time of incubation with the β-adrenoceptor blocking drug was too short to ensure a full receptor blockade. Therefore, it is doubtful whether low temperature reduces the potency of a β-adrenoceptor blocking drug. Classical criteria of receptor identification have given no evidence that the frog heart possesses α-adrenoceptors.

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