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THE FACTORS INFLUENCING THE TIME COURSE OF DRUG ACTION AT α-ADRENOCEPTORS: AN INVESTIGATION OF THE EFFECTS OF CLONIDINE IN THE PITHED RAT

J.R. DOCHERTY & J.C. McGRATH

Institute of Physiology, University of Glasgow, Glasgow G12 8QQ

1 The time courses of the pre- and post-junctional effects of clonidine were examined on heart rate and blood pressure and on the isometric tension responses of the vas deferens and anococcygeus *in situ* in the pithed rat. Plasma levels of clonidine were also monitored.

2 The time courses of the pre-junctional inhibition of sympathetic nerve mediated responses in all tissues monitored were related to the plasma levels of clonidine, but the degree of inhibition which was observed varied with the tissue and with the test stimulus employed.

3 The post-junctional agonist effects of clonidine, especially at low doses, exhibited an initial 'peak' followed by a decline to a lower plateau. This decline of the response was not due to receptor desensitization but was related to a decline in the plasma clonidine level from an initially high value caused by injection of a bolus.

4 The factors determining the time course of drugs' effects in the preparation are discussed and it is concluded that pre- and post-junctional responses should be compared at a point in time after injection of the drug, at which equilibration has occurred.

Introduction

In the pithed rat several effector systems responding to sympathetic nerve stimulation can be monitored allowing investigation of the effects of drugs on adrenergic mechanisms *in situ*. Systems which can be selectively stimulated by appropriate positioning of the pithing rod electrode include the vasopressor response of the arterial blood pressure, cardioaccelerator response of the heart (Gillespie, MacLaren & Pollock, 1970), and the isometric tension of anococcygeus (Gillespie & McGrath, 1973) or vas deferens (Gillespie & McGrath, 1974). We have now employed these preparations to investigate the pre- and post-junctional effects of the α-adrenoceptor agonist, clonidine, on adrenergic transmission. With the exception of the cardioaccelerator fibres, the adrenergic nerves to each of these tissues produce their effector response i.e. contraction of smooth muscle, via post-junctional α-adrenoceptors located on the smooth muscle. Consequently, assessment of the pre-junctional α-adrenoceptor-mediated inhibitory effect, according to the reduction of the effector response, will be complex since the pre- and post-junctional effects will be acting in physiological antagonism. In the case of the vas deferens such interpretations are further complicated by the presence of a 'non-adrenergic' nerve-induced response (Brown, McGrath & Summers, 1979).

Earlier studies employing the pithed rat have demonstrated that clonidine and other α-adrenoceptor agonists can inhibit responses to adrenergic nerve stimulation by a pre-junctional mechanism and that responses to high frequency stimulation (>1 Hz) are inhibited by a smaller proportion of their size compared with those to lower frequencies ($\leqslant 1$ Hz) (Armstrong & Boura, 1973; Drew, 1976; Doxey & Everitt, 1977). However, since responses to stimulation at $\geqslant 0.5$ Hz are restrained by endogenous activation of pre-junctional α-adrenoceptors (Docherty & McGrath, 1977b; 1979), we have analysed the effects of clonidine on responses to a single stimulus, to low frequency (0.1 Hz) or to short trains of pulses at higher frequency (in the case of the anococcygeus) to avoid activation of this endogenous α-adrenoceptor feedback system.

The pre- and post-junctional effects of clonidine have been found to possess different time courses (Drew, 1976; Doxey & Everitt, 1977; Cavero, Gomeni, Lefevre & Roach, 1978), and the dose-effect relationships obtained for actions at the two sites have not correlated well with observations made *in vitro* (Drew, 1976; 1977; Doxey & Everitt, 1977). In the present study an explanation for the time course and dose-effect relationships has been sought by relat-

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ing the pre- and post-junctional effects of clonidine on several tissues to the plasma concentration of the drug.

A preliminary account of these results has been published (Docherty & McGrath, 1978a).

Methods

Male rats were pithed by the method of Gillespie *et al.* (1970) and respired with 100% O_2 (see Clanachan & McGrath, 1976). Heart rate was extracted from right carotid arterial pressure by means of a Devices instantaneous ratemeter. Where appropriate, longitudinal isometric tension of the anococcygeus or vas deferens (Gillespie & McGrath, 1973; 1974) was monitored (Grass FTO3 transducer) and displayed together with blood pressure and heart rate on a u.v. oscillograph (S.E. Labs model 3006DL). The pithing rod electrode was placed for stimulation of the sympathetic outflows to various organs (Grass S88 stimulator, supramaximal voltage): cardiac sympathetic stimulation (C6-T1, 0.05 ms pulses); vasopressor nerves (T2-T6, 1.0 ms pulses); vas deferens (L1-L3, 0.5 ms pulses); or anococcygeus (T12-L1, 0.5 ms pulses) (Gillespie & McGrath, 1973: 1974). Gallamine (20 mg/kg) was given to prevent skeletal muscle twitching except where cardiac responses were examined, since in this latter case twitching is acceptably small (Docherty & McGrath, 1977a; 1978b). All experiments were carried out with the 10 mm long tip of the pithing rod used as an electrode.

Time course

The rates of onset of the pre- and post-junctional effects of clonidine were first tested while continuously stimulating the cardiac sympathetic nerves at 0.1 Hz. Under these conditions heart rate is elevated to a submaximal plateau, but no endogenous feedback is present (Docherty & McGrath, 1977b; 1979). When this plateau was attained, the stimulation was switched off and, after equilibrium was re-established, stimulation was repeated but this time clonidine (5 μg/kg) was injected or infused (McLennan DS 201 digital syringe) while stimulation was continued. The time course of the inhibitory effects of clonidine could therefore be compared with that of cessation of nerve activity.

To examine the duration of the effects of clonidine, several tissues were studied while applying intermittent sympathetic stimulation: cardiac sympathetic, single pulse every 60 s; (diastolic) pressor, single pulse every 2 min; vas deferens, single pulse every 5 min; anococcygeus, 1 Hz 5 pulses every 3 min. The response of the vas deferens was biphasic, with peaks occurring at 0.35 and 0.65 s (McGrath, 1978). Consequently the 2 phases were measured separately. Following clonidine, when the response became monophasic, response heights at 0.35 and 0.65 s were taken as the values of the respective phases (see Figure 4). The post-junctional agonist action of clonidine was also measured where applicable (diastolic blood pressure and anococcygeus tension). Different groups of rats were employed for examination of different tissues since the site of nerve stimulation varied.

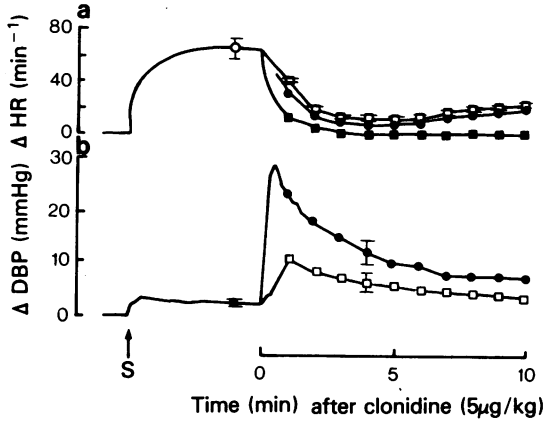

Figure 1 The rates of onset of (a) the cardiac inhibitory and (b) the pressor effects of clonidine, when injected during continuous cardioaccelerator stimulation at a frequency of 0.1 Hz. Stimulation was started at S, and clonidine (5 μg/kg) was injected (●) or infused over 60 s (□), starting at time zero. The effect of switching off the stimulator is also shown (■). The graph was constructed from the means of data taken every 10 s during rapid changes and otherwise at 1 min intervals. Control means under the three conditions were similar and were thus combined. For the sake of clarity, symbols with error bars (s.e. mean) are only shown at certain points. n = 5–6.

Site of action of clonidine

To assess whether the inhibitory effects of clonidine were pre- or post-junctional in origin, its effects on the cardiac responses to noradrenaline (200 ng/kg) were examined. To assess whether the rapid decline in the pressor response to clonidine was due to desensitization of α-adrenoceptors, the effects of an initial injection of clonidine (5 μg/kg) on the pressor effects of a subsequent injection of clonidine (5 μg/kg) or noradrenaline (200 ng/kg), given after 10 min (by which time the pressor response to the initial dose had declined), were examined.

In one group of animals, blood samples (0.5 ml) were taken from the left carotid artery at 1, 5, 15 and 40 min after clonidine injection (5 μg/kg), and plasma samples were assayed for clonidine (Draffan, Clare, Murray, Bellward, Davies & Dollery, 1976).

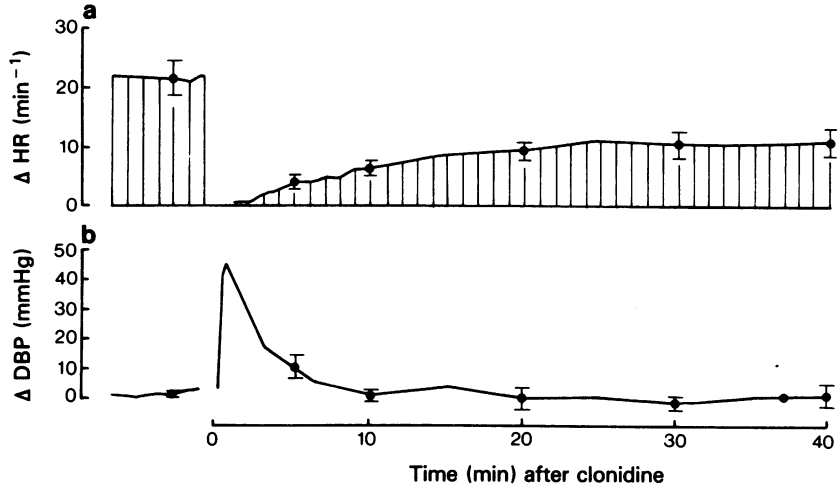

Figure 2 The rates of onset and duration of (a) the cardiac inhibitory and (b) the pressor effects, of clonidine, when injected during intermittent cardioaccelerator stimulation (1 pulse every 60 s). In (a) the vertical lines represent the height of the cardiac response to a single stimulus pulse. Clonidine (5 μg/kg) was injected at time zero. The graph was constructed from the means of data taken every 1 min, except for the initial pressor effect where the data were taken every 10 s. For the sake of clarity, symbols with error bars (s.e. mean) are only shown at certain points. $n = 6$.

Drugs used were clonidine hydrochloride (Boehringer Ingelheim), gallamine triethiodide (May & Baker), and noradrenaline bitartrate (Koch-Light).

Drugs were dissolved in saline (0.9% w/v NaCl solution). Doses quoted are in terms of the salt. Drugs were injected (or infused) in a volume of 1 ml/kg intravenously, and washed in with 1 ml/kg saline. Control saline injections were of 2 ml/kg.

Results

Time course of effects of clonidine

The rates of onset of the cardiac inhibitory and the pressor effects of clonidine (5 μg/kg) were compared by injecting the drug during continuous cardioaccelerator stimulation at a frequency of 0.1 Hz. This stimulation produced a marked cardioacceleration (Figure 1a), but only a small pressor effect (Figure 1b). When the cardioacceleration reached a steady value, clonidine (5 μg/kg) was injected. This produced a marked pressor effect which reached its maximum in less than 30 s, but had recovered to control values in less than 10 min. The cardiac inhibition was slower in onset, reaching its peak inhibition in about 4 minutes, but recovering much more slowly than the pressor response; taking 1 h or more. However, when the stimulator was simply switched off, heart rate still took about 4 min to reach control values, even though the onset of this effect was immediate. Similarly, when the stimulus pulse length was reduced from 0.05 to 0.01 ms, heart rate took 3 to 4 min to reach a new steady level. When infused over 60 s, clonidine (5 μg/kg) produced a similar inhibition of the cardioaccelerator response to that produced by rapid injection, with only a marginally slower rate of onset (Figure 1a). However, the pressor effect was much reduced with infusion as compared with injection of clonidine (5 μg/kg) (Figure 1b).

To examine more precisely the rate of onset of the cardiac inhibition, the effect of clonidine (5 μg/kg) was examined on the cardioacceleration response to a single stimulus pulse given every 60 s. These single pulses are discrete events which do not summate, so in this case there is no build up of the effector response as was the case with stimulation at 0.1 Hz. Consequently an inhibitory effect can be more precisely determined. On injection, clonidine (5 μg/kg) produced a marked pressor effect which again reached its peak in about 30 s and declined to control levels in less than 10 min (Figure 2b). Maximal inhibition by clonidine of the cardioacceleration response occurred with the first pulse which was given 1 min after injection (Figure 2a). In separate experiments, the response to a single pulse was maximally inhibited even 30 s after clonidine. The response to a single pulse did not recover to 50% of control height until 40 min after clonidine (5 μg/kg). The smallest dose of clonidine tested (0.05 μg/kg) produced a short-lived but significant inhibition, and 0.5 μg/kg reduced the 'single pulse' response from 23.8 ± 3.5 min ($n = 5$) to

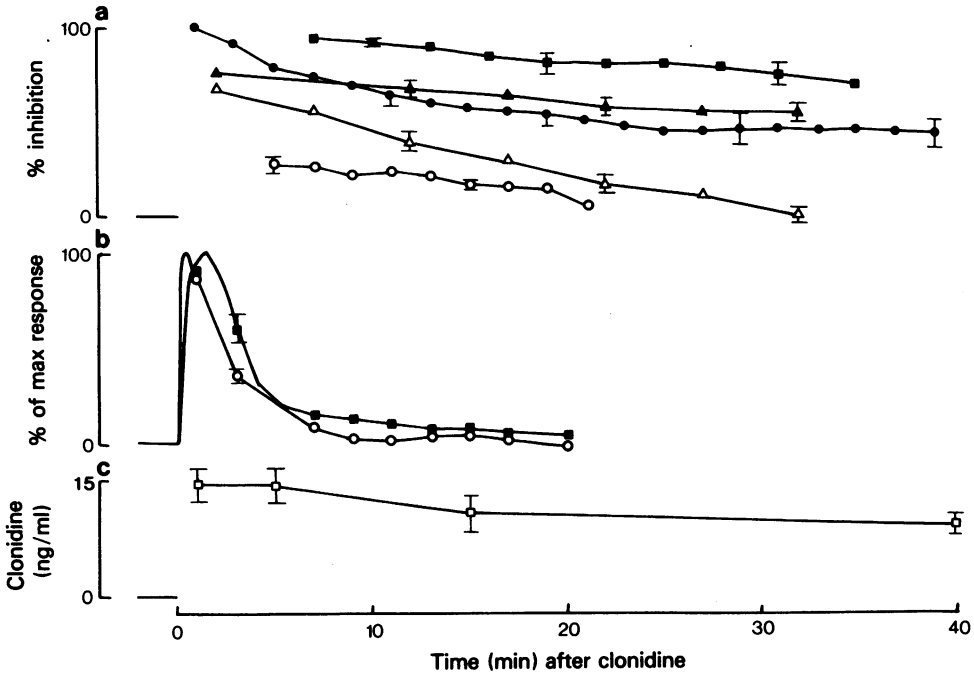

Figure 3 Graphs of the time courses of (a) the pre-junctional inhibition, (b) the post-junctional effector responses and (c) the plasma levels of clonidine, following the injection of clonidine (5 μg/kg) at time zero. Responses examined were: anococcygeus (■); vas deferens, 1st component (△), 2nd component (▲); heart rate (●); diastolic pressure (○); plasma levels (□). The graphs in (a) and (c) were constructed from the means of data taken at the points shown. In (b) graphs were constructed from the means of data taken every 10 s during rapid changes and otherwise at 1 min intervals. For the sake of clarity, symbols with error bars (s.e. mean) are only shown at certain points. n = 5–7.

11.1 ± 1.7 min after 1 min with full recovery within 15 min (see Figure 5).

The time-courses of the post-junctional effector responses and of the pre-junctional inhibition by clonidine (5 μg/kg) were examined in several tissues and related to the plasma levels of clonidine (Figure 3). Figure 3a shows the pre-junctional inhibitory effects of clonidine (5 μg/kg) expressed as percentage inhibition of the control response. The time course of recovery was slow in all cases relative to the initial post-junctional effects (Figure 3b) and the time taken to return to control values depended on the degree of the initial inhibition. Thus the pressor response to a single stimulus pulse, which 5 min after clonidine was inhibited by 27.0 ± 4.1% (n = 6) (prior to this the large pressor effect of clonidine on injection made interpretation of the nerve response difficult), recovered to control values within 25 min. The absolute size of the pressor response to a single stimulus was only 5.0 ± 0.4 mmHg (n = 6) compared with the initial response to clonidine (5 μg/kg) of over 30 mmHg (Figure 1). In contrast the anococcygeus isometric response to 5 stimuli at 1 Hz was inhibited by clonidine (5 μg/kg) by 93.8 ± 0.6% (n = 5) at 7 min and recovered only to 70% inhibition by 35 min. Prior to 7 min the effect on the nerve-induced response (absolute control value 1.88 ± 0.15 g (n = 6)) could not be accurately assessed due to the contractile effect of clonidine, e.g. peak response (90 s) 1.71 ± 0.7 g, after 5 min 0.34 ± 0.09 g (n = 6). The responses of the vas deferens and cardiac sympathetic nerves gave intermediate results. The response of the vas deferens, which was biphasic in control responses, became monophasic after clonidine. This residual response was resistant to α-adrenoceptor antagonists and thus corresponds with the 'non-adrenergic' component which has previously been described (McGrath, 1978). The first phase (peak 0.35 s) reached approximately control values by 27 min, at which time there was still no clear second phase (Figure 4). A smaller dose of clonidine (0.5 μg/kg) produced only a small inhibition of the first phase of response, with recovery by 7 min, whereas the second phase was more markedly inhibited, recovering by 22 min.

The post-junctional effects of clonidine (5 μg/kg) on both blood pressure and anococcygeus were of short

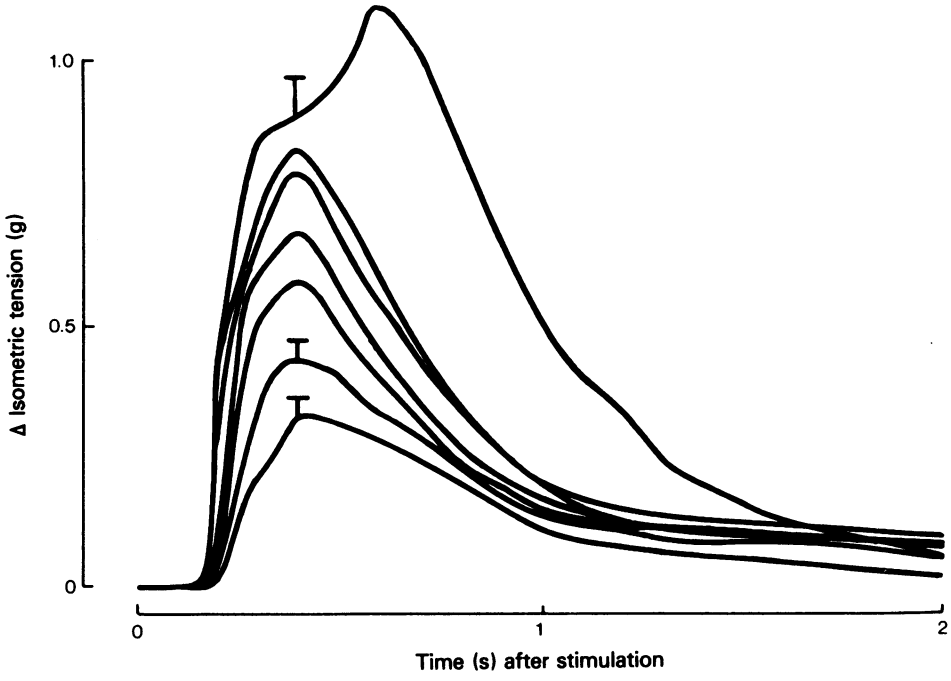

Figure 4 Effects of clonidine (5 μg/kg) on the *in situ* vas deferens in the pithed rat. The time course of the isometric tension response of the vas deferens to single pulse stimulation (L1–L3) is shown; the upper curve is the control response and the others are from bottom to top, 2 min, 7 min, 12 min, 17 min, 22 min, and 27 min after injection of clonidine. The graphs were constructed from the means of data taken every 0.1 s. For the sake of clarity, symbols with error bars (s.e. mean) are only shown at certain points. $n = 5$.

duration. In each case there was virtually complete recovery within 10 min (Figure 3b). The vas deferens was not contracted by this dose of clonidine, and no cardiac effect was found.

The plasma levels of clonidine at 1 min and at 5 min after injection of clonidine (5 μg/kg) were virtually identical. Within this same period, however, some of the effects of clonidine, particularly the post-junctional effects on the blood pressure and the anococcygeus and the pre-junctional cardio-inhibition showed a marked decline. In contrast, between 5 min and 40 min the plasma levels of clonidine showed a gradual decline which correlated well with the decline of the effects of clonidine; in particular with the more marked inhibitory effects on the heart, anococcygeus and vas deferens (Figure 3).

Site of the inhibitory effect

Noradrenaline (200 ng/kg) injected intravenously produced a cardioacceleration of 37.1 ± 6.8 min ($n = 5$). A subsequent injection of noradrenaline (200 ng/kg) given 5 min after clonidine (100 μg/kg) produced a cardioacceleration of 28.2 ± 2.3 min, and noradrenaline (200 ng/kg) injected 15 min after clonidine produced a cardioacceleration of 32.0 ± 4.0 min. In each case the response was not significantly different from the control response (Student's t test for paired data, $P > 0.05$), suggesting that the inhibitory effect even of such a large dose of clonidine was entirely pre-junctional in origin.

Origin of the decline of the post-junctional response

The last of a series of control injections of noradrenaline (200 ng/kg) produced a pressor response of 27.0 ± 5.5 mmHg ($n = 5$). Following clonidine (5 μg/kg), a subsequent dose of noradrenaline (200 ng/kg), given after the pressor response to clonidine had disappeared, produced a pressor effect of 29.9 ± 6.6 mmHg which was not significantly different from the control response (Student's t test for paired data).

On injection, clonidine (5 μg/kg) produced a pressor effect of 39.6 ± 6.6 mmHg ($n = 6$). A subsequent injection of clonidine (5 μg/kg) given 10 min later, after the pressor response to the first dose had disappeared, produced a pressor effect of 40.9 ± 7.6

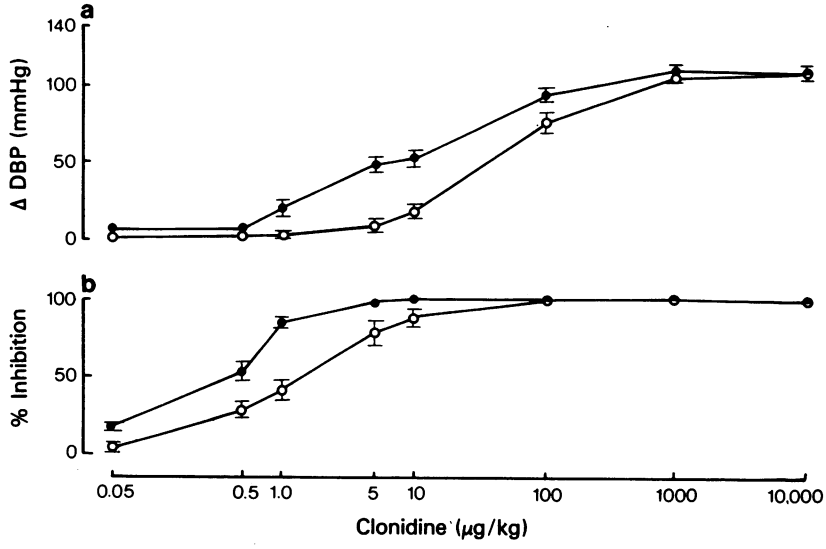

Figure 5 Comparison of the dose-response curves for (a) the post-junctional pressor response to clonidine and (b) the pre-junctional inhibition by clonidine of the cardiac sympathetic response to a single stimulus pulse. The peak response to clonidine (●) was compared with the response 5 min after injection of clonidine (○). Error bars represent s.e. mean. n = 5–7.

mmHg, which was not significantly different from the response to the first dose (Student's t test for paired data). This reproducibility of pressor responses to clonidine and noradrenaline suggests that the fall off in the pressor response is not due to receptor desensitization.

Comparison of dose-response curves for pre- and post-junctional effects

Since the 'peak' responses to clonidine gave a 'non-equilibrium' estimate of the potency of clonidine, the pressor response and the inhibition of the cardiac sympathetic response to a single stimulus pulse were compared at their respective peaks and at 5 min after injection. When plotted in the latter manner, each dose-response curve was shifted to the right (Figure 5). When each parameter was measured (a) at the 'peak' or (b) at 5 min, the ratio between the pre- and post-junctional effects of clonidine was similar (Table 1) and was equivalent to that found *in vitro* in rabbit pulmonary artery (Starke, Montel, Gayk & Merker, 1974). Comparing the peak with the equilibrium (5 min) effects, the dose-response curves showed a 4 to 5 fold shift in each case and comparing the pre- and post-junctional effects, a difference of 20 to 30 fold was found (Table 1).

Table 1 Relative potency of clonidine at pre- and post-junctional α-adrenoceptors: peak response and response 5 min after injection

	Peak response (μg/kg)	*5 min response (μg/kg)*	*Dose ratio (5 min/peak)*
Pressor effect (dose producing rise in DBP of 50 mmHg)	7.08	33.9	4.8
Cardiac inhibition (ID_{50})	0.30	1.26	4.2
Dose-ratio (DBP/HR)	23.6	26.9	1.14

Values obtained from regression analysis of the linear part of each curve in Figure 5.

This demonstrates the need to make quantitative comparisons at equivalent times. For example if the initial pre-junctional effect was not perceived due to the protocol employed (e.g. Figure 1) then the initial pressor effect, which would appear on the recording trace, might be compared with the cardio-inhibitory effect at 5 min; this would give a pre-: post-junctional 'dose-ratio' of 5.6 instead of 23 or 29 (see Table 1).

Discussion

These results confirm that, in the pithed rat, the net effects from pre- and post-junctional actions of clonidine can have different time-courses (Cavero *et al.*, 1978). However, the time courses of each action can be related to the concentration of clonidine at the tissue and to the frequency-response characteristics of the effector system. Especially with small doses, initial effects of short duration and related to the initial high concentration caused by bolus injection are found. This emphasizes the need for careful analysis of data obtained from different tissues *in situ* when comparing the different types of effects of drugs administered via the cardiovascular system. The time at which the effects are measured becomes critical for several reasons.

First, the route and rate of injection will determine the distribution of the drug e.g. before equilibrium is attained an initial high concentration will course through the vascular system. If administration is intravenous then the heart and arterial systems will sample this high concentration rapidly, and the concentration sampled by other tissues will differ according to their blood supply. This initial high concentration lasts for only a part of the first 60 s after injection since the maximum inhibitory effect of clonidine has occurred by this time and since the plasma levels of clonidine have, at 1 min, equilibrated to a value which is still maintained at 5 min. Within this initial 60 s the concentration of drug in arterial blood will have been subject to a wide variation starting from a sudden high level as the venous injectate, subject only to mixing in the cardio-pulmonary circulation, makes its 'first-pass' through the arterial system; on returning to the venous system, mixing with blood will be almost complete while equilibration with extra-vascular fluid may take somewhat longer depending on the chemical properties of the drug and the blood supply to the particular tissue. This can be exemplified by the results of injecting clonidine (5 μg/kg) intravenously. The injectate has a concentration of clonidine of 5000 ng/ml. After 60 s the plasma concentration of clonidine is 15 ng/ml. Even assuming that the injectate (0.25 ml in a 250 g rat) was diluted by 30 times (i.e. in 7.5 ml = approximately half the blood volume of a 250 g rat) in its passage to the left side of the heart, the 'first pass' arterial concentration would be approximately 166 ng/ml or 11 times the final equilibrium level. The transient passage of such a concentration would produce a concentration of drug at the appropriate receptors which, while transient, would initiate effects which would last longer in a slowly adapting effector system. Thus the 'peak' effects of a drug may be reliably 'dose-related' but comparisons between organ systems, differently situated receptors in the same organ or between different drugs will be subject to several qualifications. This interpretation is supported by the reduction in the initial pressor effect brought about by replacing the intravenous bolus with a slow infusion: a further corollary which was found in three rats was that the initial pressor effect could be *increased* by administering clonidine into the aorta via the carotid artery thereby preventing mixing in the cardio-pulmonary system (authors, unpublished observations).

Secondly, the elimination of the drug will determine whether and for how long an equilibrium throughout the body may be obtained.

In the case of a drug such as noradrenaline which is rapidly eliminated, the major opportunity for agonist activity will thus come during the initial 'bolus' phase while a drug such as clonidine, which is more slowly eliminated, will appear to certain tissues in an initial high concentration and subsequently, after equilibration, will be distributed evenly throughout the body. This factor becomes critical when comparing the effects of chemically dissimilar adrenoceptor agonists (authors, unpublished observations).

Thirdly, the rate at which the effector tissue is capable of responding to the action of the drug is critical. For example, an effect due to contraction of smooth muscle can rapidly follow receptor activation, dependent only on the rate of contraction of the particular tissue e.g. post-junctional α-adrenoceptor agonism on resistance vessels or anococcygeus. In contrast an inhibitory effect exerted pre-junctionally, but which is being monitored according to the post-junctional effector response, will be dependent for its onset on the reduction in the effector response. Where the effector response is due to a single stimulus, the accuracy of measurement of the time course of an inhibitory effect will depend on how frequently the individual stimuli can be delivered without summation of the effects. In contrast, when continuous nerve stimulation produces a maintained response, the 'inhibitory' effect can only be gauged by comparing the fall in response produced by the drug with that which would occur by ceasing nerve stimulation. Both of these cases were illustrated by the inhibitory effects of clonidine (5 μg/kg) against the cardioaccelerator response. The importance of this factor became particularly apparent when the effects of smaller or larger doses of clonidine were examined in an attempt to produce a dose-response

relationship for the pre-junctional inhibitory effect against cardioaccelerator stimulation at 0.1 Hz. In this situation, cessation of electrical stimulation of the cardioaccelerator nerves returned the heart rate to control levels within 3 to 4 min. Following injection of clonidine (5 μg/kg) during continuous stimulation, the consequent reduction in heart rate reached a maximum at 3 to 4 min. However, with smaller doses of clonidine (0.05 to 0.5 μg/kg) the fall in heart rate reached a peak within 3 min and then started to recover towards the pre-injection level. In the latter situation, therefore, the effect of the drug was on the wane before its full extent could be indicated by the effector system. For the time course of the inhibitory effect of low and moderate doses of clonidine, therefore, intermittent stimulation with single pulses (e.g. 1 pulse per 30 s) gives a more accurate result. On the other hand, if the dose-response relationship to high doses of clonidine is to be established, it is necessary to monitor the effect against continuous stimulation or intermittent trains of stimuli at low frequency (e.g. 0.1 Hz). Higher frequencies ($\geqslant$0.5 Hz) should be avoided since endogenous activation of the α-adrenoceptor feedback loop will occur (Docherty & McGrath, 1977b; 1979 and unpublished observations). Similarly the onset and termination of post-junctional effects will play a role in determining the apparent dose-response relationship with the 'peak' effects. For example, as the dose of clonidine increased, the time at which the 'peak' effects were attained on both blood pressure and anococcygeus became later, reflecting the time necessary for the smooth muscle to contract in response to the initial high concentration of agonist (see above). In the case of anococcygeus, *in vivo* or *in vitro*, the tension induced by noradrenaline or by nerve stimulation can take several minutes to reach a plateau (Gillespie, 1972; Gillespie & McGrath, 1973); this may be one reason why the 'peak' effect of a bolus injection of clonidine occurs later with anococcygeus than with blood pressure. A further consequence is that the decline in the initial response will reflect the rate at which the smooth muscle relaxes after drug-induced contraction rather than the (faster) rate at which the concentration of drug declines. Hence the apparent contradiction between equal plasma clonidine levels yet different response heights at 1 and 5 min; at 1 min, clonidine levels have equilibrated but muscle tension has not recovered from the effect of the initial bolus.

Fourthly, the possibility of physiological antagonism between effects of the drug occurring at different sites, and which may have different time courses, will distort the net effect monitored. In the case of clonidine this particularly applies to post-junctional α-adrenoceptor agonism which will alter the baseline from which nerve-mediated effects (and their inhibition) are measured. This is most marked in tissues such as resistance vessels and anococcygeus which are sensitive to post-junctional α-adrenoceptor agonists (Gillespie, 1972; McGrath, 1973); their susceptibility to the pre-junctional inhibitory effects of clonidine could only be assessed after the decline of the initial post-junctional agonist effect. The vas deferens is less susceptible to sustained contraction by α-adrenoceptor agonists (Boyd, Chang & Rand, 1960). Nevertheless care is still required with this tissue since a variety of depolarizing stimuli including the α-adrenoceptor agonists noradrenaline, phenylephrine and clonidine can potentiate nerve-induced contractions at doses which are sub-contractile (Sjöstrand & Swedin, 1974; Brown *et al.*, 1979, A. McDonald & J. C. McGrath, unpublished observations). In the heart, where the adrenergic response is mediated via β-adrenoceptors, post-junctional α-agonism is less important. However, in this case high doses of clonidine (1 mg/kg) produced a post-junctional depression of the accelerator response to noradrenaline which tends to exaggerate the inhibitory effect of clonidine against cardioaccelerator nerve stimulation.

Considering together these four factors, the time courses and dose-response relationships for clonidine and other agonist drugs can be explained as follows. On intravenous injection, clonidine will rapidly pass in a high concentration through the heart, resistance vessels and tissues receiving a profuse blood supply. This 'first-pass' effect will result in immediate, dose-related effects including contraction of smooth muscle (post-junctional) and inhibition of any sympathetic-nerve induced responses which are subsequently initiated. After equilibration of the drug between blood and tissues, the concentration of clonidine at the receptors will fall to a lower level which will then decline relatively slowly at a rate inversely related to dose and related to metabolism and excretion of the drug. Since lower concentrations of clonidine are required for pre- than for post-junctional effects (Starke *et al.*, 1974) a considerable difference may thus appear between the initial post-injection and the subsequent equilibrium periods. Thus at low concentrations of clonidine ($\leqslant$10 μg/kg), both pre- and post-junctional effects appear rapidly on injection (when the monitoring system is capable of showing this) while only the pre-junctional effect remains after equilibration; the concentration of clonidine having fallen below the threshold for post-junctional α-adrenoceptor agonism. The alternative explanation of receptor desensitization can be eliminated by the ability of a subsequent injection of clonidine or noradrenaline to reproduce their control pressor effects. As the dose of clonidine increases, the concentration remaining at the receptors during the equilibrium phase increases to levels above the threshold for post-junctional effects and hence the pressor effects persist longer

(Figure 5). A similar relationship occurred with the contraction by clonidine of the *in situ* anococcygeus but in this latter case the particular sensitivity to the post-junctional effect prevented quantitative analysis of the initial pre-junctional effects.

From the above discussion it is clear that there are considerable advantages in measuring the effects of a drug such as clonidine at some point where equilibrium exists, e.g. 5 min, rather than at the 'peak'. Nevertheless, when examining the effects of a drug with a relatively short half-life it may be necessary to take the 'peak' as the only realistic estimate of potency.

As noted above, the post-junctional α-agonism by clonidine produced responses in the various tissues monitored which would be expected from their known properties. The inhibitory effect, however, varied between tissues and, in the case of the vas deferens, even varied between the two components of the response. The order of decreasing susceptibility to the inhibitory effect of clonidine was anococcygeus, vas second phase, cardiac sympathetic, vas first phase and pressor nerves. The relative susceptibility of the cardiac and resistance of the pressor response are of particular interest in view of the use of clonidine as an anti-hypertensive agent. Significant inhibition of the cardioaccelerator response was produced by clonidine (0.05 to 0.5 μg/kg). These doses are approaching those used clinically (Frisk-Holmberg, Edlund & Paalzow, 1978). The particular susceptibility of the anococcygeus to the inhibitory effects of clonidine confirms the *in vitro* observation that concentrations as low as 10^{-9} M were effective against contractile responses to field stimulation (Idowu & Zar, 1976). The relative susceptibility to clonidine of the second adrenergic component of the vas deferens contractile response compared with the first 'non-adrenergic' component (McGrath, 1978) has also been noted *in vitro* (Brown *et al.*, 1979). A clearer separation of the two components can also be achieved *in vitro* or *in vivo* by separately recording the contraction of the two ends of the tissue since one component dominates in each end. Nevertheless the relative susceptibility to clonidine of the adrenergic component to stimulation of the spinal outflow in the present study reinforces the *in vitro* observations. The similarity between the *in vitro* and pithed rat effects of clonidine on anococcygeus and vas deferens also indicate no detectable effect at the ganglion synapse.

Although the study concentrated on clonidine, the factors discussed above will apply to the effects of a wide variety of pharmacological agents tested on the pithed rat. This has been demonstrated for other adrenoceptor agonists when comparing their relative potencies at various receptors but will also apply, for example, to the interpretation of experiments involving drugs which modify the effects of agonists (authors, unpublished observations).

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