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LACK OF CORRELATION BETWEEN PRESYNAPTIC INHIBITION OF NORADRENALINE RELEASE AND END ORGAN RESPONSES DURING NERVE STIMULATION

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1 LD 3098 (cirazoline) is an imidazoline derivative, possessing agonist properties at α-adrenoceptor sites.

2 When transmitter release was measured directly as tritium overflow from perfused cat spleen preparations, prelabelled with [^{3}H]-noradrenaline, LD 3098 was found to be 10 times more selective for presynaptic than for postsynaptic α-adrenoceptors.

3 In addition, in this preparation, LD 3098 appears to induce a postsynaptic sensitization to the transmitter released by nerve depolarization because under conditions in which [^{3}H]-noradrenaline overflow decreased, there was a paradoxical potentiation in the response to nerve stimulation. This potentiation also occurred with a concentration of LD 3098 that did not *per se* affect stimulation-evoked [^{3}H]-noradrenaline release or the basal perfusion pressure of the spleen.

4 Both the reduction in ^{3}H-transmitter release induced through activation of α-presynaptic adrenoceptors and the potentiation of the responses to nerve stimulation were concentration-dependent phenomena.

5 In pentobarbitone anaesthetized dogs, the heart rate response to low frequency ansa-subclavia stimulation was not affected by LD 3098. Whilst the α_1 mediated increase in blood pressure responses to injected noradrenaline and tyramine was significantly potentiated by LD 3098, the β_1-mediated heart rate responses to these injected amines were not modified in the presence of LD 3098.

6 Thus it is possible that the failure to detect any presynaptic effects with LD 3098 when transmitter release is measured indirectly at the level of the postsynaptic responses is due to end organ sensitivity changes.

7 These findings emphasize that caution is necessary when assessing presynaptic α-adrenoceptor effects through end organ responses to nerve stimulation both *in vitro* and *in vivo* and the need for measurements of transmitter overflow as well as adequate postsynaptic controls in such experiments.

Introduction

Presynaptic α-adrenoceptors located at the terminal varicosities of sympathetic neurones regulate, by a negative feedback mechanism, the release of noradrenaline induced by nerve depolarization (Langer, 1974; 1977; 1978; Starke, 1977; Westfall, 1977). The presence of this mechanism in tissues receiving sympathetic innervation has been observed both *in vitro* (Enero, Langer, Rothlin & Stefano, 1972; Starke, 1972) and *in vivo* (Armstrong & Boura, 1973; Drew, 1976; Doxey & Everitt, 1977; Yamaguchi, De Champlain & Nadeau, 1977; Roach, Lefevre & Cavero, 1978; Steppeler, Tanaka & Starke, 1978), indicating a physiological role for these release-modulating presynaptic α-adrenoceptors.

However, in many studies, especially those *in vivo*, the assessment of the affinity of drugs for presynaptic α-adrenoceptors has been indirect and based on measurement of changes in end organ response, especially heart rate (Armstrong & Boura, 1973; Drew, 1976; Doxey & Everitt, 1977; Roach *et al.*, 1978; Constantine, Weeks & McShane, 1978). In some cases this has been done with little, if any, attention being paid to alterations in sensitivity of the end organ responses after drug treatment despite the early paper by Boissier, Giudicelli, Fichelle, Schmitt & Schmitt (1968) which demonstrates that clonidine modifies the blood pressure response to injected noradrenaline in anaesthetized dogs.

We now describe some observations with an imidazoline derivative having α-adrenoceptor agonist activity, LD 3098 (cirazoline), a potent nasal vasoconstrictor, which demonstrate the difficulties in inter-

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preting results when end organ response alone is used as an index of effects on transmitter release.

Methods

In vitro *studies*

Perfused cat spleen. Cats of either sex (1.5 to 2.5 kg) were used and anaesthetized with pentobarbitone (30 mg/kg i.p.) After evisceration, the spleen was isolated and the splenic artery and vein were cannulated. A pair of fine platinum electrodes was hooked around the splenic artery in order to stimulate the postganglionic nerve fibres. The spleen was placed in a plethysmograph filled with liquid paraffin kept at 37°C. The tissue was perfused with Krebs solution at 37°C, at a constant flow rate of 7.5 ml/min. Changes in perfusion pressure were measured with a Palmer pressure recorder.

The composition of the Krebs solution was as follows (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.6, $MgCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 25.0, glucose 11.1, Na_2EDTA 0.004 and ascorbic acid 0.11. The solution was bubbled continuously with a mixture of 95% O_2 and 5% CO_2 and kept at 37°C. Stimulation was carried out with an S-44 Grass stimulator; square pulses of 0.5 ms duration and of supramaximal voltage were used.

Ten min after the supramaximal voltage was determined, (−)-[7-^{3}H]-noradrenaline (Radiochemical Centre, Amersham; specific activity 11 Ci/mmol) was infused for 10 min at a rate of 5 μCi/min. After the infusion of [^{3}H]-noradrenaline was completed, the spleen was perfused for 60 min before the periods of nerve stimulation were begun.

In every experiment five periods of nerve stimulation were applied at 30 min intervals and 300 shocks at a frequency of 2 Hz were delivered during each period. After two control stimulation periods, LD 3098 was added to the perfusion medium in increasing concentrations 22 min before the ensuing period of nerve stimulation. When the effect of LD 3098 was tested in the presence of phentolamine (3.1 μM) the α-blocking agent was added 30 min before the first stimulation period and was present for the remainder of the experiment.

Samples of the splenic effluent were collected at 1 min intervals before and during each period of stimulation followed by three consecutive 1 min samples in the poststimulation period. The samples were centrifuged to remove the red blood cells and aliquots of the supernatant were assayed for total tritium by liquid scintillation spectrometry.

The spleen, at the end of each experiment, was blotted on filter paper, weighed and homogenized in 10 ml of 0.4 N perchloric acid per g of tissue. The homogenate was centrifuged and an aliquot of the supernatant was assayed for total tritium. The total overflow of radioactivity elicited by nerve stimulation was expressed as fractional release per shock (Dubocovich & Langer, 1976).

Statistical calculations were performed by conventional procedures (Snedecor & Cochran, 1967).

In vivo *studies*

The studies were conducted on mongrel dogs (13 to 17 kg body weight). Animals were anaesthetized with pentobarbitone (35 mg/kg i.v.) and a constant level of anaesthesia maintained by the infusion of 6 mg kg^{-1} h^{-1} pentobarbitone into a femoral or brachial vein.

After intubation and bilateral vagotomy, the chest was opened on the right side at the second intercostal space and the animals respired with a Bird Mark 8 respirator. Shielded platinum electrodes were then placed around the right ansa subclavia and cardiac postganglionic fibres were submaximally excited using a Grass stimulator (S44 or S88) at 10 V, 1 ms with frequencies up to 2 Hz for 1 min periods.

In most experiments the preganglionic superior cervical nerve to the nictitating membrane was stimulated at submaximal frequencies (up to 2 Hz at 10 V and 1 ms for 1 min periods) and contractions of the membrane were recorded by means of a Grass FT03C transducer. Blood pressure was measured from a femoral artery with a Statham P23Db transducer and the pressure pulse used to trigger a heart rate meter. All recordings were made on Grass model 7 D polygraphs.

Drugs were infused into a femoral vein according to the following protocol. At least 30 min were allowed to elapse following preparation of the animal and the experiment started when the measured parameters were stable. Animals were first subjected to 1 min periods of ansa subclavia stimulation (AS) and nictitating membrane stimulation (NMS). On completion of these procedures, injections of the following drugs were made in amounts causing submaximal effects, every 3 to 5 min, depending on the duration of the responses: noradrenaline (NA, 1 to 10 μg), tyramine (Tyr, 0.5 to 1 mg) and angiotensin II (Ang, 1 to 2 μg). All drug solutions were prepared in 0.9% w/v NaCl solution (saline).

After completion of two series of control stimulations as described above, and only if responses were similar in the two control series, clonidine or LD 3098 was infused at 0.3 μg kg^{-1} min^{-1} intravenously. A higher infusion rate, 3 μg kg^{-1} min^{-1}, was then employed with clonidine so as to produce a similar increase in blood pressure to that observed with LD 3098. Drug infusions lasted 30 to 60 min. and stimulation and injection of reference drugs was started when the measured parameters had plateaued.

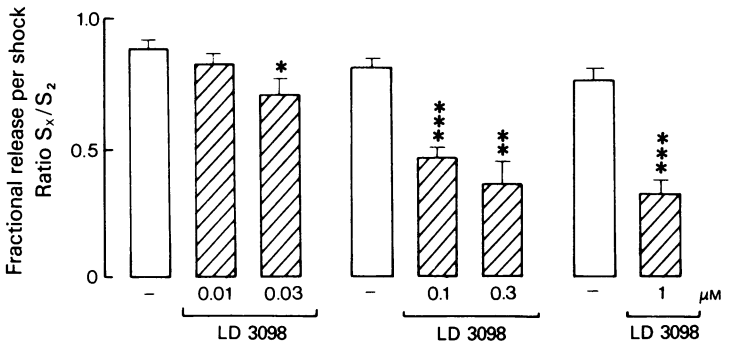

Figure 1 Effect of different concentrations of LD 3098 on ^{3}H-transmitter overflow elicited by nerve stimulation from the perfused cat spleen. Ordinate scale: fraction of the total tissue radioactivity released per shock expressed as the ratio obtained between each period of nerve stimulation (Sx) and the corresponding control (S_2) within each experiment. The nerves were stimulated at 2 Hz during 150 s (0.5 ms duration, supramaximal voltage). LD 3098 in the concentrations indicated, was added 22 min before the corresponding period of nerve stimulation. Open columns: controls; hatched columns: LD 3098 (Cirazoline). Mean values are shown of at least 3 experiments per group; vertical lines show s.e. means $*P < 0.05$; $**P < 0.005$; $***P < 0.001$ when compared with the corresponding controls.

Results are expressed as changes from control values (second series) in heart rate, nictitating membrane tension or in systolic and diastolic blood pressure. Clonidine and LD 3098 increase blood pressure and nictitating membrane tension whilst reducing heart rate, and the changes shown in the following tables are measured from the new stable, post drug levels. Initial control values for blood pressure and heart rate in the two groups of dogs used in this study are given in Table 1; the initial resting nictitating membrane tension being set at 10 g in all preparations. In the dog experiments all drug concentrations are expressed as the salt.

Table 1 Effects of clonidine and LD 3098 infusions in the dog

Treatment		*Systolic blood pressure* (mmHg)	*Heart rate* (beats/min)	*Contraction of the nictitating membrane* (g)
Control values		142 ± 13	133 ± 15	Resting tension = 10 g
Clonidine	Maximum change at 0.3 μg kg^{-1} min^{-1} i.v.	+11 ± 10	−29 ± 16	+1.1 ± 0.3
	Maximum change at 3 μg kg^{-1} min^{-1} i.v.	+48 ± 10*	−36 ± 22	+8.3 ± 2.7
Control values LD 3098		148 ± 12	122 ± 10	Resting tension = 10 g
	Maximal change at 0.3 μg kg^{-1} min^{-1} i.v.	+49 ± 12*	−6 ± 2	+9.3 ± 1.5

Control values for blood pressure and heart rate (means ± s.e. mean of 4 or 5 experiments) in the two groups of dogs used for the infusion studies. Also shown are the maximum changes from these control values in systolic blood pressure, heart rate and nictitating membrane tension obtained during the infusions of clonidine or LD 3098. Resting tension of the nictitating membrane was set at 10 g.

* $P < 0.05$ when compared to the respective control value (paired Student's t test).

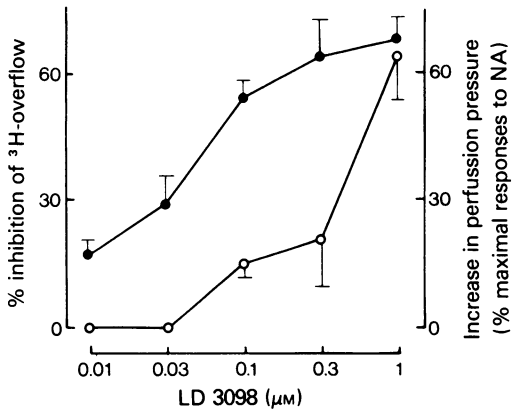

Figure 2 Differences in activity of LD 3098 on pre- and postsynaptic α-adrenoceptors of the perfused cat spleen. Left ordinate scale: (●) presynaptic effect expressed as % inhibition of ^{3}H-transmitter overflow induced by nerve stimulation in the presence of different concentrations of LD 3098. The nerves were stimulated at 2 Hz for 150 s (0.5 ms duration, supramaximal voltage). Right ordinate scale: (○) postsynaptic effect. Increase in the perfusion pressure in the presence of different concentrations of LD 3098 expressed as % of the maximal response to noradrenaline (maximal response to NA: 270 ± 34.3 mmHg, $n = 4$). Abscissa scale: micromolar concentration. LD 3098 in the concentrations indicated was added 22 min before the corresponding period of nerve stimulation. The perfusion pressure was measured before each period of nerve stimulation when the maximal increase was obtained for each concentration of LD 3098. NA: (−)-noradrenaline; LD 3098: cirazoline. Mean values of at least 3 experiments per group are shown; vertical lines indicate s.e. mean.

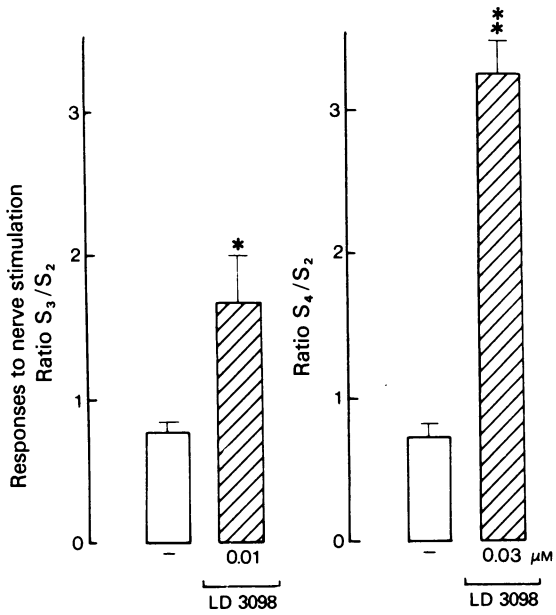

Figure 3 Potentiation by LD 3098 of the postsynaptic responses induced by nerve stimulation in the perfused cat spleen. Ordinate scale: responses induced by nerve stimulation (2 Hz for 150 s, 0.5 ms, supramaximal voltage) expressed as the ratio obtained between the period of nerve stimulation in which LD 3098 was present (S_3, S_4) and the corresponding control (S_2) within each experiment. Open columns: controls; hatched columns: LD 3098 (cirazoline). Mean values of at least 3 experiments per group are shown; vertical lines show s.e. mean. $^*P < 0.05$; $^{**}P < 0.001$ when compared with the corresponding controls.

Drugs

The following drugs were used: pentobarbitone sodium (Abbott); (−)-noradrenaline bitartrate (Sigma); tyramine hydrochloride (Sigma); angiotensin II (Ciba); phentolamine methanesulphonate (Ciba); clonidine hydrochloride (Boehringer); LD 3098 (Cirazoline, 2-[2-cyclopropylphenoxylmethyl]-2-imidazoline hydrochloride), synthesized by Drs H. Najer and P. Guidicelli in the Chemistry Department of Synthélabo.

Results

In vitro *studies*

In the perfused cat spleen the fraction of the total tissue radioactivity released by nerve stimulation at 2 Hz was: 7.9 ± 1.9 ($\times 10^{-5}$), $n = 7$. Under control conditions this value did not decline during five consecutive periods of nerve stimulation.

LD 3098 produced a statistically significant, concentration-dependent decrease in stimulated ^{3}H-overflow from the cat spleen over the concentration range 0.03 to 1 μM (Figure 1), but it did not affect the spontaneous efflux of radioactivity from the spleen.

A selective stimulation of presynaptic α-adrenoceptors with no post-synaptic activation was achieved with 0.03 μM of LD 3098. This concentration produced a significant reduction (29 ± 6%, $n = 3$, $P < 0.05$ when compared with controls) in stimulated ^{3}H-transmitter release, with no change in spleen perfusion pressure (Figure 2). Higher concentrations of LD 3098 did produce increases in spleen basal perfusion pressure and these increases were dose-related.

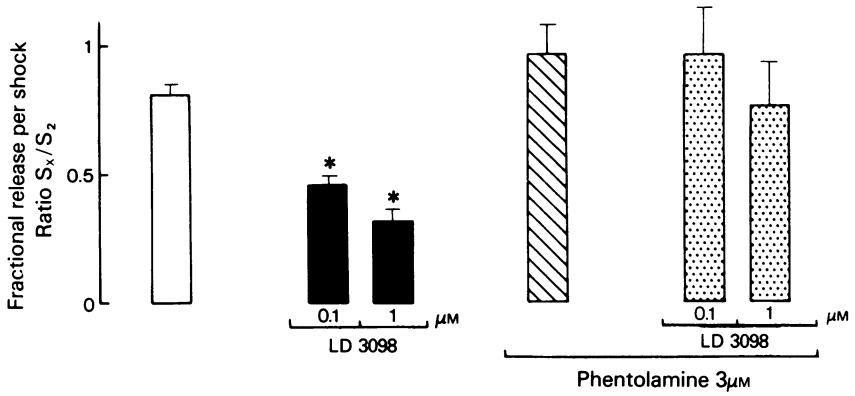

Figure 4 Effect of LD 3098 on ^{3}H-transmitter overflow elicited by nerve stimulation in the presence of phentolamine in the perfused cat spleen. Ordinate scale: fraction of the total tissue radioactivity released per shock expressed as the ratio obtained between the period of nerve stimulation (Sx) and the corresponding control (S_2) within each experiment. The nerves were stimulated at 2 Hz for 150 s (0.5 ms duration, supramaximal voltage). The absolute values of fractional release per shock during the second period of nerve stimulation at 2 Hz were: $7.93 \pm 1.89 (\times 10^{-5})$, $n = 7$ in controls and $34.70 \pm 12.32 (\times 10^{-5})$, $n = 4$ in the presence of 3.1 μM phentolamine. Open columns: control; solid columns: LD 3098; hatched column: phentolamine 3.1 μM; stippled column: phentolamine 3.1 μM plus LD 3098. LD 3098 in the concentrations indicated was added 22 min before the corresponding period of nerve stimulation. Phentolamine 3.1 μM was added 30 min before the first period of nerve stimulation and remained present throughout the experiment. Mean values of at least 4 experiments per group are shown; Vertical lines indicate s.e. mean. $*P < 0.001$ when compared with the corresponding control.

Nevertheless in concentrations of 0.03 μM to 0.3 μM, LD 3098 induced a selective stimulation of presynaptic α-adrenoceptors although at 1 μM the inhibition of ^{3}H-release had reached a plateau ($68 \pm 5\%$, $n = 5$) whilst the postsynaptic effects measured as spleen perfusion pressure changes increased steeply (Figure 2).

Despite this marked reduction in transmitter release, LD 3098 increased, in a concentration-dependent manner, the responses to nerve stimulation. Figure 3 shows the potentiation observed with 0.01 and 0.03 μM of LD 3098. These concentrations of the α-agonist did not modify the basal perfusion pressure of the spleen and only at 0.03 μM did LD 3098 inhibit the nerve stimulation induced transmitter release (Figure 2). A potentiation of the responses to nerve stimulation was also observed over the concentration range 0.1 to 1 μM, under conditions in which LD 3098 increased the basal perfusion pressure by direct activation of postsynaptic α-adrenoceptors.

The α-adrenoceptor antagonist, phentolamine (3.1 μM), increased the release of [^{3}H]-noradrenaline and blocked the responses induced by nerve stimulation in the perfused cat spleen by blocking the pre and postsynaptic α-adrenoceptors respectively. When phentolamine (3.1 μM) was perfused from 30 min before the first stimulation, the decline in overflow of total tritium, as a result of four consecutive periods of nerve stimulation was small when these values were expressed with reference to the second period of nerve stimulation. The LD 3098-induced inhibition of the stimulated [^{3}H]-noradrenaline release was abolished in the presence of phentolamine (Figure 4), providing evidence that LD 3098-inhibition of neurotransmission is probably mediated through activation of presynaptic α-adrenoceptors. In addition the increase in the basal perfusion pressure induced by LD 3098, as well as the responses to nerve stimulation, were blocked by this concentration of phentolamine (data not shown).

In vivo *studies*

Figure 5 shows a typical experimental trace obtained on infusion of LD 3098 at 0.3 $\mu g\,kg^{-1}\,min^{-1}$ intravenously. At this infusion rate LD 3098 produced an increase in systolic and diastolic blood pressure of 35 to 40 mmHg which reached a plateau approximately 15 min after starting the infusion. LD 3098 also induced a contraction of the nictitating membrane which developed slowly and plateaued some 40 min after starting the LD 3098 infusion. During the initial phases of the infusion of LD 3098, potentiation of the nictitating membrane response to nerve stimulation occurred (Figure 5a), but this effect was not evident 40 to 50 min later when the contraction developed by

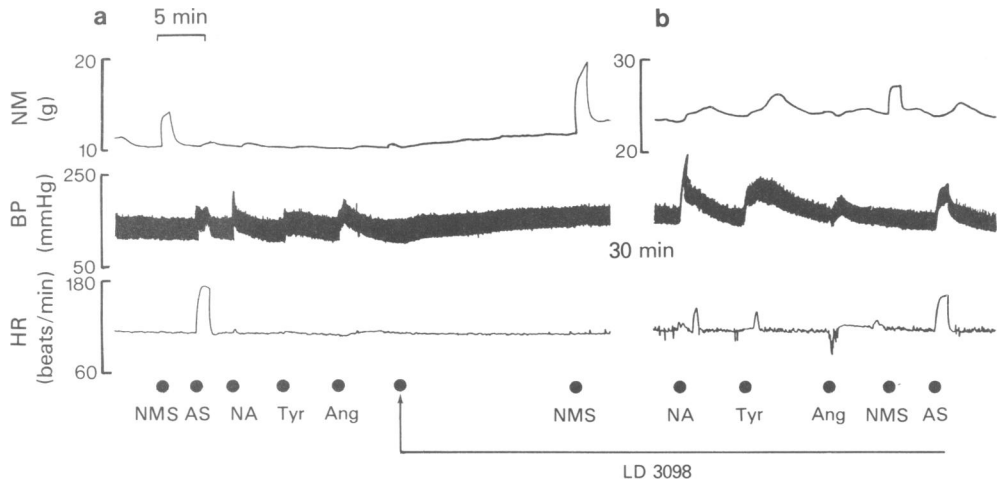

Figure 5 Anaesthetized dog preparation: the effect of an infusion of LD 3098 on blood pressure, heart rate and nictitating membrane tension. Ordinates: Top trace: nictitating membrane (NM) tension (g). Middle trace: blood pressure (BP) (mmHg); Bottom trace: heart rate (HR) (beats per min). NA: noradrenaline, 5 μg; Ang: angiotensin II, 1 μg; Tyr: tyramine, 0.25 mg; AS: Ansa subclavia stimulation (10 V, 1 Hz, 1 ms for 1 min); NMS: nictitating membrane stimulation (10 V, 1 Hz, 1 ms for 1 min). Representative trace of a typical experiment. Between the first and second parts of the figure 30 min were allowed for stabilization of the NM contraction to LD 3098. LD 3098 infusion at 0.3 μg kg^{-1} min^{-1} i.v. for remainder of the experiment. Note the change in scale of the nictitating membrane trace between (a) and (b).

the nictitating membrane was considerably larger than at the beginning of the infusion of LD 3098 (Figure 5b). In fact in the group of animals studied, the contractions of the nictitating membrane to stimulation of the superior cervical preganglionic nerve were not significantly different in the presence of LD 3098 when compared to the control period (Table 2). Some potentiation of the nictitating membrane response to injected noradrenaline and tyramine is evident from Figure 5b but these changes did not reach levels of statistical significance because they were often masked by increases in base line elicited by

Table 2 Effect of infusions of clonidine and LD 3098 on heart rate increases and nictitating membrane contractions induced by sympathetic nerve stimulation

Treatment	*Ansa stimulation* ΔHR *(beats/min)*	*Nictitating membrane* $\Delta tension$ *(g)*
Control response	50 ± 4	5.1 ± 0.4
Clonidine 0.3 μg kg^{-1} min^{-1}	44 ± 2	3.6 ± 1.1
Clonidine 3 μg kg^{-1} min^{-1}	18 ± 9*	1.4 ± 0.8**
Control response	66 ± 6	4.2 ± 0.7
LD 3098 0.3 μg kg^{-1} min^{-1}	63 ± 10	3.7 ± 1.0

Control values for the increase, over baseline levels, in heart rate or nictitating membrane tension induced by sympathetic nerve stimulation (10 V, 1 ms, 1 to 2 Hz for 1 min) and the effects on such stimulations of infusions of either clonidine or LD 3098. The stimulation frequencies used were submaximal and the values shown are means ± s.e. mean of 4 experiments. Pre-treatment control levels and the baseline levels for heart rate and nictitating membrane tension during clonidine or LD 3098 infusions are given in Table 1.
* $P < 0.05$; ** $P < 0.01$ when compared to the appropriate control value (paired Student's t test).

LD 3098. Whilst the heart rate response to ansa subclavia stimulation was slightly reduced during LD 3098 infusion in the experiment shown in Figure 5(a-b), overall the mean response of the group of animals infused with LD 3098 was not significantly different from control values (Table 2). Figure 5b also shows the potentiation induced by LD 3098 of the blood pressure responses to ansa stimulation, noradrenaline and tyramine and the fact that, by contrast, those to angiotensin II were not significantly affected (Table 3).

Whilst the α_1 mediated blood pressure increases to injected noradrenaline and tyramine were markedly potentiated by LD 3098, the effect of LD 3098 on the β_1 mediated heart rate responses to these injected amines, were not consistently potentiated. The heart rate increases elicited by noradrenaline and tyramine were 28 ± 9 and 10 ± 4 beats/min respectively before LD 3098 and during the infusion of LD 3098, 23 ± 7 and 19 ± 4 beats/min.

As can be seen in Table 1, systolic blood pressures and heart rates were similar in the two groups of dogs used for the clonidine and LD 3098 infusion studies, blood pressure being about 145 mmHg and heart rate around 130 beats/min. Clonidine produced dose-related increases in systolic blood pressure and nictitating membrane tension while it reduced the heart rate (Table 1). LD 3098, like clonidine, increased systolic blood pressure and nictitating membrane tension but the fall in heart rate with LD 3098 was less pronounced (Table 1). LD 3098 at 0.3 $\mu g\,kg^{-1}\,min^{-1}$ produced approximately equivalent increases in blood pressure and nictitating membrane tension to 3 $\mu g\,kg^{-1}\,min^{-1}$ clonidine.

Table 2 shows the effect of the two different infusion rates of clonidine on ansa stimulation, and on responses of the nictitating membrane to preganglionic nerve stimulation. Clonidine produced a decrease in the heart rate to ansa stimulation and reduced the effect of nictitating membrane stimulation, both in a dose-dependent manner.

In Table 3 it can be seen that the clonidine infusion produced small increases in the systolic blood pressure responses to noradrenaline, tyramine and angiotensin-II and that this effect was more marked with the higher rate of infusion. Only the systolic blood pressure increase to tyramine was significantly potentiated by clonidine and this at the higher infusion rate (Table 3). There was no significant potentiation by clonidine of the diastolic blood pressure responses to any of the injected reference drugs, except in the case of angiotensin-II but this was not a dose-related effect, occurring only with the lower clonidine infusion rate (Table 3).

Unlike clonidine, LD 3098 did not reduce the effects of ansa stimulation or inhibit the effects of nictitating membrane stimulation despite marked increases in the basal tension of the membrane at least equivalent to that induced by the high infusion rate of clonidine (Table 2). Noradrenaline and tyramine systolic and diastolic blood pressure responses were significantlly potentiated by LD 3098 but there was only a small increase in the response to angiotensin-II which was not statistically significant (Table 3).

Discussion

LD 3098 is an imidazoline derivative possessing direct, α-agonist properties. Unlike clonidine, it is not

Table 3 Effects of infusions of clonidine and LD 3098 on the systolic and diastolic blood pressure responses to injected noradrenaline, tyramine and angiotensin II

		Increases in blood pressure (mmHg)					
		Noradrenaline		*Tyramine*		*Angiotensin II*	
Treatment		Sys	Dias	Sys	Dias	Sys	Dias
Control response		41 ± 4	23 ± 3	28 ± 8	25 ± 5	35 ± 5	31 ± 3
Clonidine	0.3 $\mu g\,kg^{-1}\,min^{-1}$	59 ± 9	30 ± 5	45 ± 15	28 ± 8	40 ± 4	44 ± 6*
	3 $\mu g\,kg^{-1}\,min^{-1}$	61 ± 10	40 ± 13	55 ± 7*	30 ± 5	46 ± 16	43 ± 17
Control response		49 ± 4	29 ± 4	30 ± 4	16 ± 1	30 ± 4	30 ± 7
LD 3098	0.3 $\mu g\,kg^{-1}\,min^{-1}$	115 ± 19*	46 ± 6*	81 ± 15*	44 ± 8**	45 ± 16	40 ± 10

Maximum increases in systolic (Sys) and diastolic (Dias) blood pressure, above baseline levels, induced by intravenous injections of submaximal doses of noradrenaline, (1 to 10 μg); tyramine (0.2 to 1 mg) and angiotensin II (1 to 2 μg), before and during the infusion of either clonidine or LD 3098. Values shown are the means ± s.e. mean of 4 or 5 experiments. Pre-treatment control levels for blood pressure and the baseline level during clonidine or LD 3098 infusions are given in Table 1.

*$P < 0.05$, **$P < 0.01$ when compared to the appropriate control value (paired Student's t test).

sedative and does not induce centrally mediated hypotension when administered intravenously to anaesthetized cats (Lefèvre, Depoortere & Cavero, 1976; Lefèvre, Fenard & Cavero, 1977; Roach, Boudot & Cavero, 1977). In the studies reported here on vagotomized, anaesthetized dogs, infusions of LD 3098 were observed to increase blood pressure and to contract the nictitating membrane; properties consistent with the compound being an α-agonist. In the perfused spleen of the cat LD 3098 also increased perfusion pressure, an effect that was blocked by the α-adrenoceptor antagonist phentolamine.

With most *in vivo* studies the assessment of presynaptic α-adrenoceptor activity is carried out by stimulating the sympathetic nerves to the heart usually in the rat or the dog. In this tissue the fact that β-adrenoceptors predominate postsynaptically permits a simple, indirect assessment of presynaptic α-adrenoceptor agonist activity, by measuring the cardiodecelerator effect, preferably in spinal or ganglion blocked preparations so as to avoid reflex changes in heart rate. Since postsynaptic activation of α-adrenoceptors in vascular smooth muscle *in vivo* induces contraction, increases in blood pressure or hind limb perfusion pressure are often used as the index of postsynaptic activity in these studies (Drew, 1976; Roach *et al.*, 1977; Steppeler *et al.*, 1978).

Some of these experiments become difficult to interpret when it is not known if the end organ response elicited by nerve stimulation undergoes changes in sensitivity during the experiment. For this reason it is advisable to combine such studies with ones where transmitter release is measured directly on nerve stimulation either as noradrenaline or as ^{3}H-overflow after prelabelling of the sympathetic neurones with [^{3}H]-noradrenaline (Enero *et al.*, 1972; Starke, 1972; Dubocovich & Langer, 1974; Langer, Dubocovich & Celuch, 1975). That this caution is advisable is evident from the findings reported here with LD 3098.

In the cat spleen prelabelled with [^{3}H]-noradrenaline, LD 3098 did not affect basal efflux of tritium in concentrations up to 1 μM but reduced, in a concentration-dependent manner, the [^{3}H]-noradrenaline overflow induced by sympathetic nerve stimulation. This presynaptic activity of LD 3098 on neuronal α-adrenoceptors was selective at 0.03 μM since this concentration of drug did not affect spleen basal perfusion pressure. Increases in basal perfusion pressure, an index of postsynaptic α-adrenoceptor activity, were observed with higher doses of LD 3098 and were also concentration dependent. That activation of both pre- and postsynaptic α-adrenoceptors is accomplished by LD 3098 was confirmed by the fact that phentolamine blocked these responses in the perfused cat spleen. Several reports have indicated that in a given tissue the presynaptic α-adrenoceptors that regulate the release of noradrenaline during nerve stimulation differ in their affinity for agonists and antagonists from the postsynaptic α-adrenoceptors that mediate the responses of the effector organ (Dubocovich & Langer, 1974; Langer, 1974; Starke, Endo & Taube, 1975). In the perfused cat spleen, LD 3098 was found to be about 10 times more potent in reducing [^{3}H]-noradrenaline release during nerve stimulation than in stimulating the postsynaptic α-adrenoceptors. A similar difference in relative affinities for the pre- and postsynaptic α-adrenoceptors has been found with oxymetazoline in this preparation (Dubocovich & Langer, unpublished observations). These results are compatible with the view that in the perfused cat spleen, LD 3098 is a selective presynaptic α-adrenoceptor agonist, comparable to oxymetazoline which is known to be one of the most selective α-presynaptic agonists (Starke *et al.*, 1975). However, despite marked reductions in [^{3}H]-noradrenaline overflow to nerve depolarization in the presence of LD 3098, this compound paradoxically increased the postsynaptic response to nerve stimulation. The postsynaptic sensitization observed with LD 3098 was dose-dependent and occurred with low concentrations of the drug which did not *per se* activate either pre- or postsynaptic α-adrenoceptors. It is of interest to note that with oxymetazoline there was no potentiation of the end organ response to nerve stimulation in the perfused cat spleen (Dubocovich and Langer, unpublished observations).

Because of this profile of action in the cat spleen it was of interest to study the effects of such an α-adrenoceptor agonist *in vivo*. We decided to examine LD 3098 on the heart rate response of anaesthetized dogs to ansa stimulation and use this as the index of presynaptic α-adrenoceptor agonist activity while the effect on blood pressure was used as a measure of postsynaptic α-adrenoceptor activity. The *in vivo* study demonstrated that LD 3098 was some ten times more potent than clonidine at the postsynaptic α-adrenoceptors located in the nictitating membrane and in vascular smooth muscle. In addition LD 3098 produced a smaller decrease of basal heart rate than that obtained with clonidine infusions. Furthermore clonidine, unlike LD 3098, reduced responses of the heart and nictitating membrane to sympathetic nerve stimulation, indicating stimulation of presynaptic inhibitory α-adrenoceptors subserving a modulator role at sympathetic neuroeffector junctions (Drew, 1976; Doxey & Everitt, 1977; Steppeler *et al.*, 1978). Attention should be drawn to the fact that the nictitating membrane contracts after clonidine and consequently baseline increases could mask the effects of nerve stimulation. Indeed Tsai, Langer & Trendelenburg (1967) have demonstrated that in the spinal cat the response to injected noradrenaline decreases in relation to the resting tone of the nictitating membrane. Infusions of noradrenaline causing less than 15% of

the maximum tone produce small potentiations of the response to injected noradrenaline but the response to injected amine decreases as the degree of tone increases, until at 75% maximum tone, the response is abolished. Under our experimental conditions it is unlikely that baseline changes in nictitating membrane tension are completely masking the effects of nerve stimulation in light of the findings with LD 3098 which contracted the membrane to approximately the same extent as clonidine (9.3 ± 1.5 g for $0.3\ \mu g\ kg^{-1}\ min^{-1}$, LD 3098; 8.3 ± 2.7 g for $3\ \mu g\ kg^{-1}\ min^{-1}$, clonidine). Yet under these experimental conditions LD 3098 did not affect the response of the nictitating membrane to nerve stimulation, while clonidine produced a significant inhibition.

The above results *in vivo* would suggest that LD 3098 was devoid of presynaptic α agonist effects and that it is a postsynaptic α agonist of high selectivity. However at the same time, even during its own α agonist response, LD 3098 potentiated blood pressure responses to both noradrenaline and tyramine. Consequently in light of the perfused cat spleen results it is possible that the failure to demonstrate a presynaptic α-adrenoceptor agonist effect indirectly in the heart and in the nictitating membrane, is due to the fact that the end organ responses were potentiated despite a reduction in transmitter release. With clonidine, where no significant alteration in blood pressure response to noradrenaline was observed, it was possible to obtain evidence *in vivo* consistent with presynaptic α-adrenoceptor activation in both the heart and the nictitating membrane. At the present time it is not possible to ascribe the lack of effect of LD 3098 on ansa stimulation to such postsynaptic sentitivity changes occurring in cardiac muscle, since we have not directly studied transmitter release in the canine heart. The finding in these studies that heart rate changes elicited by exogenous sympathomimetic amines were less affected by LD 3098 than blood pressure responses may indicate that the potentiation is due to vascular rather than cardiac muscle sensitization and evidence from the rat heart would also support this contention (Roach *et al.*, 1978).

As to the mechanism of the potentiation of postsynaptic responses by LD 3098, *in vivo* responses mediated via α-adrenoceptors appear more susceptible than responses mediated via β-adrenoceptors or angiotensin-II receptors. Further studies are underway in order to clarify the mechanism of this sensitization. This property of LD 3098 may prove to be advantageous for long lasting local vasoconstriction as for instance in the nasal mucosa.

In summary, the results of these studies indicate the importance of direct measurement of transmitter release and that it is necessary to perform adequate postsynaptic controls when transmitter-release is being estimated indirectly, especially after drug treatment which could result in changes in end-organ sensitivity. The present results demonstrate a striking property of the imidazoline derivative, cirazoline: marked sensitization of the smooth muscle responses mediated by postsynaptic α-adrenoceptors both *in vitro* and *in vivo*.

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References

ARMSTRONG, J.M. & BOURA, A.L.A. (1973). Effects of clonidine and guanethidine on peripheral sympathetic nerve function in the pithed rat. *Br. J. Pharmac.*, **47**, 850–852.

BOISSIER, J.R., GIUDICELLI, J.F., FICHELLE, J., SCHMITT, H. & SCHMITT, H. (1968). Cardiovascular effects of 2-(2,6 dichlorophenylamino)-2 imidazoline hydrochloride (St 155). *Eur. J. Pharmac.*, **2**, 333–339.

CONSTANTINE, J.W., WEEKS, R.A. & MCSHANE, W.K. (1978). Prazosin and pre-synaptic α-receptors in the cardio-accelerator nerve of the dog. *Eur. J. Pharmac.*, **50**, 51–60.

DOXEY, J.C. & EVERITT, J. (1977). Inhibitory effects of clonidine on responses to sympathetic nerve stimulation in the pithed rat. *Br. J. Pharmac.*, **61**, 559–566.

DREW, G.M. (1976). Effects of α-adrenoceptor agonists and antagonists on pre- and post-synaptically located α-adrenoceptors. *Eur. J. Pharmac.*, **36**, 313–320.

DUBOCOVICH, M.L. & LANGER, S.Z. (1974). Negative feedback regulation of noradrenaline release by nerve stimulation in the perfused cat's spleen: differences in potency of phenoxybenzamine in blocking pre- and post-synaptic adrenergic receptors. *J. Physiol.*, **237**, 505–519.

DUBOCOVICH, M.L. & LANGER, S. Z. (1976). Influence of the frequency of nerve stimulation on the metabolism of ^{3}H-norepinephrine released from the perfused cat spleen: differences observed during and after the period of stimulation. *J. Pharmac. exp. Ther.*, **198**, 83–101.

ENERO, M. A., LANGER, S. Z., ROTHLIN, R.P. & STEFANO, F.J.E. (1972). Role of the α-adrenoceptor in regulating noradrenaline overflow by nerve stimulation. *Br. J. Pharmac.*, **44**, 672–688.

LANGER, S.Z. (1974). Presynaptic regulation of catecholamine release. *Biochem. Pharmac.*, **23**, 1793–1800.

LANGER, S.Z. (1977). Presynaptic receptors and their role in

the regulation of transmitter release. *Br. J. Pharmac.*, **60,** 481–497.

LANGER, S.Z. (1978). Modern concepts of adrenergic transmission. In *Neurotransmitter Systems and their Clinical Disorders.* ed. Legg, N.J., pp. 29–51, London: Academic Press.

LANGER, S.Z., DUBOCOVICH, M.L. & CELUCH, S.M. (1975). Prejunctional regulatory mechanisms for noradrenaline release elicited by nerve stimulation. In *Chemical Tools in Catecholamine Research.* ed.. Almgren, C., Carlsson, A. & Engel, J. Vol II, pp 183–191. Amsterdam: Elsevier, North Holland.

LEFEVRE,, F., DEPOORTERE, H. & CAVERO, I. (1976). Studies on LD 3098, a new nasal vasconstrictor of imidazoline series. *Fedn Proc.*, **35,** 286.

LEFEVRE, F., FENARD, S. & CAVERO, I. (1977). Vascular β-adrenoceptor stimulating properties of phenylephrine. *Eur. J. Pharmac.* **43,** 85–88.

ROACH, A.G., BOUDOT, J.P. & CAVERO, I. (1977). Centrally mediated cardiovascular effects of LD 3098 a nasal vasoconstrictor. *Fedn Proc.*, **36,** 955.

ROACH, A.G., LEFEVRE, F. & CAVERO, I. (1978). Effects of prazosin and phentolamine on cardiac pre-synaptic α-adrenoceptors in the cat, dog and rat. *Clin. exp. Hypertension,* **1,** 87–102.

SNEDECOR, G. W. & COCHRAN, W. G. (1967). *Statistical Methods.* 6th ed. Ames Iowa: Iowa State University Press.

STARKE, K. (1972). Alpha sympathomimetic inhibition of adrenergic and cholinergic transmission in the rabbit heart. *Naunyn-Schmiedebergs Arch Pharmac.*, **274,** 18–45.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. *Rev. Physiol. Biochem. Pharmac.*, **77,** 1–124.

STARKE, K., ENDO, T. & TAUBE, H.D. (1975). Relative pre- and postsynaptic potencies of α-adrenoceptor agonists in the rabbit pulmonary artery. *Naunyn-Schmiedebergs Arch. Pharmac.*, **291,** 55–78.

STEPPELER, A., TANAKA, T. & STARKE, K. (1978). A comparison of pre- and postsynaptic α-adrenergic effects of phenylephrine and tramazoline on blood vessels of the rabbit *in vivo. Naunyn Schmiedebergs Arch. Pharmac.*, **304,** 223–230.

TSAI, T.H., LANGER, S.Z. & TRENDELENBURG, U. (1967). Effects of dopamine and α-methyl dopamine on smooth muscle and on the cardiac pacemaker. *J. Pharmac. exp. Ther.*, **156,** 310–324.

WESTFALL, T.C. (1977). Local regulation of adrenergic neurotransmission. *Physiol. Rev.*, **57,** 659–728.

YAMAGUCHI, N., DE CHAMPLAIN, J. & NADEAU, R.A. (1977). Regulation of norepinephrine release from cardiac sympathetic fibers in the dog by presynaptic α- and β-receptors. *Circulation Res.*, **41,** 108–117.

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