Br. J. Pharmac. (1985), **85**, 737–746

Simultaneous measurement of contractile effects in the circular and longitudinal smooth muscle of the rat vas deferens by drugs perfused externally or via the lumen

Pedro A. Busatto & Aron Jurkiewicz

Department of Pharmacology, Escola Paulista de Medicina, Caixa Postal 20372, 01000 Sao Paulo, Brazil

1 The effects of noradrenaline and barium chloride were studied in the rat isolated vas deferens by perfusion of drugs either externally or through the lumen of the organ. Two effects were recorded simultaneously in the same preparation: (a) isometric contractions, due to the tension elicited by drugs on the external (longitudinal) smooth muscle layer and (b) pressure of internal perfusion, due to contractions of the internal (circular) smooth muscle layer.

2 It was found with the longitudinal muscle that: (a) the potency, expressed as pD_2 values, and the maximum response to noradrenaline were lower if the drug was perfused internally rather than externally; (b) the differences in maximum effects were pronounced on the prostatic half but were not observed on the epididymal half; (c) the maximum response obtained by internal perfusion could be increased by simultaneously adding the same dose of drug externally; (d) when barium chloride was used instead of noradrenaline no significant differences were observed on pD_2 values, but differences on maximal responses were similar to that observed for noradrenaline; (e) it was possible to block completely the effect of internal or external noradrenaline on the longitudinal muscle, by perfusing external phenoxybenzamine. In these conditions the responses of the circular muscle to the agonist were only partly blocked.

3 With the circular muscle, the differences related to internal and external perfusion were less marked than in the longitudinal muscle. However, unlike the latter, the circular layer was slightly more sensitive to drugs applied internally, in relation to pD_2 values.

4 It is suggested that the difference in pD_2 values may be due to the removal of noradrenaline by the neuronal uptake process, whereas the difference in maximal effect is due to the inaccessibility of part of the receptor population when drugs are added through the lumen.

Introduction

Since the earliest reports on the pharmacology of the vas deferens (Martins & Valle, 1939) studies have been conducted mainly on the contractility of the longitudinal muscle; only recently attention has been drawn to the circular layer (Anstey, 1971; Anstey *et al.*, 1974; Jackson & Tomlinson, 1978; Anstey & Birmingham, 1978; 1980). The present studies were undertaken in order to obtain futher information about the pharmacological properties of the latter layer. Besides considering its contractile characteristics, we thought it interesting to analyse the circular layer as a diffusion barrier, as drugs added to the lumen have to cross it to reach the longitudinal muscle. In other words, it was also used here as a tool for the study of the role of drug diffusion on receptor mechanisms.

One of the fundamental postulates of receptor theory is that pharmacological receptors (Clark, 1933) are homogeneously distributed in a kinetic compartment, the biophase (Furchgott, 1955). The penetration of a drug into the biophase has been mostly compared, experimentally and theoretically, to the crossing of a single diffusion barrier (Furchgott, 1955), although some extensions to that model have been suggested (Paton & Waud, 1964; Pton & Rang, 1965; Thron & Waud, 1968; Furchgott, 1972; Jurkiewicz *et al.*, 1973).

Notwithstanding its usefulness as a pharmacological tool, the model of a single biophase barrier can hardly be correlated with the histological conformation of a smooth muscle preparation. In fact, if a drug is added to an organ chamber, its molecules have to diffuse through non-contractile as well as contractile tissues in order to reach all the elements of a given population of receptors. For instance, contractions of the rat vas deferens, as usually recorded, are

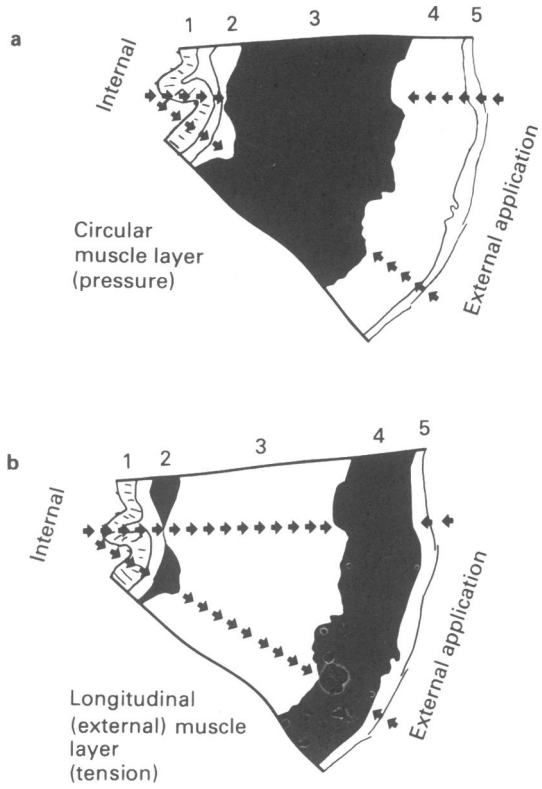

Figure 1 Cross-sectional diagram indicating the location of circular and longitudinal smooth muscle layers in the wall of the rat vas deferens. Dark areas represent the muscle layer causing respectively the increase of lumen pressure (a), or of longitudinal tension (b). The arrows indicate the diffusion that has to be undergone by the drug in order to reach the pharmacological receptors, when applied internally or externally. Numbers indicate: (1) mucosa, (2) scattered longitudinal smooth muscle bundles (in guinea-pig vas deferens, these bundles do not contribute to the longitudinal tension (Anstey & Birmingham, 1980), (3) circular smooth muscle layer, (4) longitudinal smooth muscle layer and (5) serosa. The circular layer is thicker at the prostatic than at the epididymal end (not shown).

assumed to be due to a longitudinal smooth muscle layer in which receptors are supposed to be evenly distributed. This layer represents about one fourth to one third of the thickness of the organ wall, and is externally located, in relation to a bulky circular layer. The circular layer is practically equidistant in relation to the inner and outer surfaces of the vas deferens (Figure 1a). On the other hand, the distance between the longitudinal layer and the external surface of the organ is much shorter than the corresponding distance to the lumen (Figure 1b). As a consequence, if a drug is applied to the organ bath, it has to cross only the connective tissue constituting the serosa, in order to reach the longitudinal layer, but if perfused through the lumen it has to cross the mucosa plus the circular smooth muscle. Therefore from the standpoint of the biophase model, internal and external application can be pictured as two alternative paths for the drug to reach a given receptor population. Consequently, in the present study we have attempted to verify whether there are differences in the contractile characteristics of the longitudinal and circular smooth muscle layers when drugs are applied externally or in the lumen of the vas deferens. Preliminary communications of parts of the present work have been published previously (Busatto & Jurkiewicz, 1976; 1977; Jurkiewicz *et al.*, 1978).

Methods

Animals

Adult albino rats, weighing 280–350 g were killed by inhalation of an overdose of ether. The vas deferens was excised and cleaned of surrounding tissues and of its luminal secretion, in order to be used for the perfusion procedure. In some experiments the vas deferens was cut into epididymal and prostatic halves.

Perfusion system

The glass chamber (diameter: 0.4 cm and length: 9.0 cm) was maintained at 30°C by means of heated water circulating between double walls. A lateral inlet was present at the bottom of the chamber for the entrance of external perfusion fluid. The outlet and drain for this fluid were located about 6.0 cm above the inlet. At its lower extremity, the organ chamber was closed by a small rubber stopper perforated by a 10-gauge stainless steel cannula. This cannula was introduced about 0.5 cm into the lumen of the vas deferens, at the prostatic end, and firmly tied to it. It served as an inlet for the internal perfusion fluid. Another 10-gauge cannula, curved at its upper end, was introduced at the epididymal end of the vas deferens. It was used as an outlet for the internal perfusate.

A non-pulsatile 'polystaltic' pump (Buchler, mod. 2-6100) was used. Internal and external perfusion were performed at the same flow rate (2 ml min^{-1}) through two independent channels of the pump.

A tension transducer (Hewlett-Packard, mod FTA-101) was linked to the epididymal cannula via a hook whereas a pressure transducer (Hewlett-Packard, mod 267-BC) was linked, by means of a side-arm, to the tube conducting the internal perfusion fluid. Transducers were connected to a Statham recorder through Hewlett-Packard amplifiers.

Perfusion of the vas deferens

Continuous internal and external perfusion were performed simultaneously, with an aerated nutrient solution (mM): NaCl 138, KCl 5.7, $CaCl_2$ 1.8, NaH_2PO_4 0.36, $NaHCO_3$ 15, glucose 5.5, prepared in glass-distilled, deionized water. Two independent reservoirs were used so that drugs could be added for either internal or external perfusion.

In general, the first dose was given after an equilibration period of 30–60 min, and was washed out after 5 min. There was about 30 min between two consecutive drug perfusions. Internal and external drug perfusions could be performed alternately in the same preparation. In some of the experiments drugs were added simultaneously to the internal and external perfusion solutions.

Measurement of drug effects

Contractions of longitudinal smooth muscle were measured as g (tension). Pressure of internal perfusion was recorded as mmHg. The values of pD_2 were calculated as the negative logarithms of the dose of drug inducing a 50% effect (ED_{50}). Significance of differences was analysed using the *t* test (Snedecor & Cochran, 1967).

Measurements of radioactivity

After internal or external perfusion of radioactive noradrenaline (up to 10^{-4} M, with an activity of about 4×10^4 c.p.m.), samples of 0.5 ml of internal and external perfusion fluids were added to vials containing 10 ml Insta-gel (Packard Instrument Company,

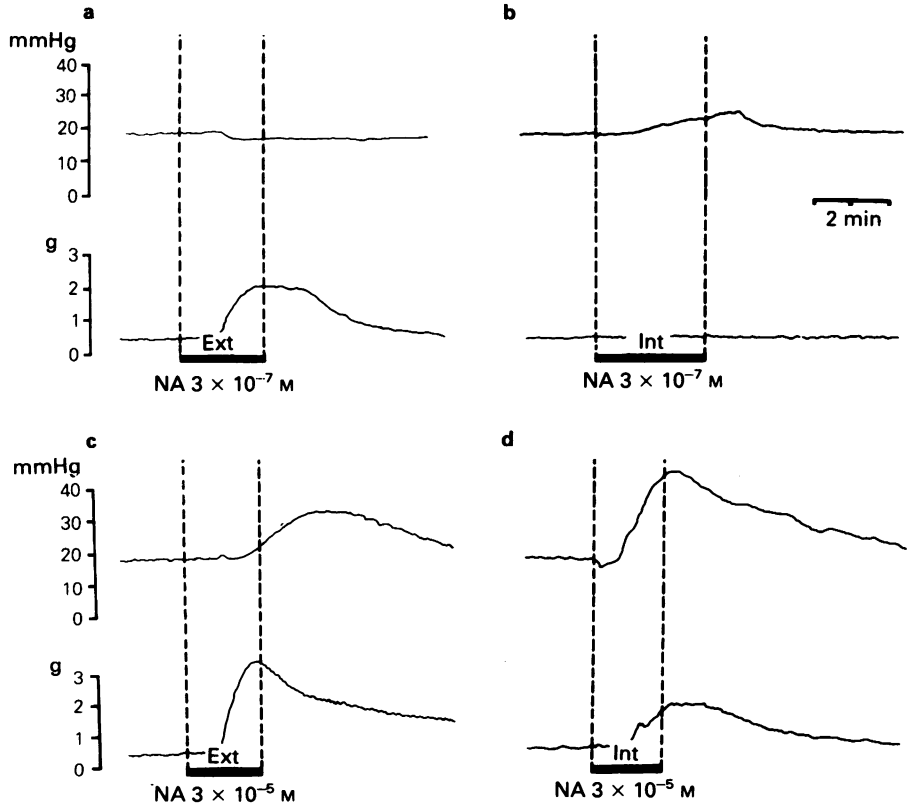

Figure 2 Effects of 3×10^{-7} M (a,b) and 3×10^{-5} M (c,d) noradrenaline (NA) added to the external nutrient solution (Ext), at (a) and (c), and to the internal nutrient solution (int), at (b) and (d). Pressure of internal perfusion was recorded (mmHg), indicating the contractions of the circular smooth muscle layer. Tension (g), elicited on the longitudinal layer, was recorded simultaneously. Horizontal bars and vertical dotted lines indicate the period during which NA was present in the perfusing solutions.

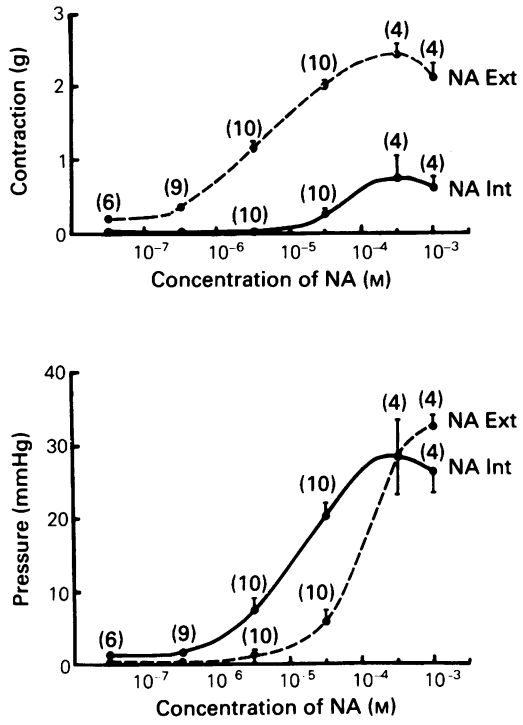

Figure 3 Log dose-response curves obtained from experiments similar to that shown in the previous figure, in which the longitudinal tension and the pressure of internal perfusion were simultaneously recorded during addition of noradrenaline to the internal (NA Int, solid line) or external (NA Ext, broken line) perfusion fluid. Number of experiments are in parentheses, vertical bars indicate s.e.mean. Maximal effects for tension, but not for pressure, were significantly different between NA Int and NA Ext ($P<0.01$).

Inc.) and the radioactivity read in a Beckman LS-100 scintillation counter.

Drugs

(−)-Noradrenaline ((−)-arterenol hydrochloride, Sigma, U.S.A.), barium chloride (May and Baker, U.S.A.), phenoxybenzamine HCl (SK & F, USA) and (−)-[7-^{3}H (N)]-noradrenaline (0.05 μCi ml^{-1}), from New England Nuclear, U.S.A., were used. Working solutions were prepared shortly before the experiments, from stock solutions, Ascorbic acid (6×10^{-5} M) was added to noradrenaline solution to prevent oxidation.

Results

Characteristics of the contractile effects of noradrenaline

Figure 2 shows the effects of a small dose (3×10^{-7} M) and a large dose (3×10^{-5} M) of noradrenaline, during external or internal perfusion. The small dose, when added externally (a), induced a contraction of the longitudinal fibres, without increasing the pressure. On the other hand, when added internally (b), it induced a contraction of the circular fibres without affecting tension. These results are in accordance with the expectations based on the histological distribution of smooth muscle fibres in the rat vas deferens, as shown in Figure 1, namely that longitudinal fibres are externally whereas circular fibres are internally located. Our results also show that longitudinal and circular layers can contract independently from each other. In addition, when the large dose of noradrenaline was added externally (c) or internally (d), the

Table 1 Maximal effects (E_{max}) and pD_2 values on circular and longitudinal muscle layers after internal and external perfusion of noradrenaline or barium chloride

Agonist	*Route of perfusion*	E_{max} *Longitudinal* g	% [a]	*Circular* mmHg	% [a]	pD_2 *Longitudinal* pD_2	Dose-ratio [b]	*Circular* pD_2	Dose-ratio [b]
Noradrenaline	External	2.42 ± 0.17	100	32.5 ± 1.3	100	5.57 ± 0.20	1	4.01 ± 0.21	1
	Internal	0.72 ± 0.30**	30	28.3 ± 5.4	87	4.06 ± 0.07**	32	4.62 ± 0.11*	0.25
Barium chloride	External	4.29 ± 0.23	100	103.9 ± 18.6	100	1.78 ± 0.12	1	1.17 ± 0.17	1
	Internal	1.25 ± 0.23**	29	57.8 ± 8.4*	56	1.54 ± 0.12	1.7	1.42 ± 0.13	0.56

Values are given ± s.e.mean

[a] % maximal effect in relation to maximal effect by external perfusion, for the same muscle layer and agonist.

[b] Ratio between ED_{50} value and ED_{50} obtained by external perfusion, for the same muscle layer and agonist.

Value significantly different from that obtained by external perfusion: *$P<0.05$; **$P<0.01$.

respective contraction of the external or internal smooth muscle layer was immediately followed by a contraction of the adjacent layer. This contraction was interpreted as being due to the diffusion of the drug through the wall of the organ, reaching progressively both muscle layers.

Experiments similar to that shown in Figure 2 were repeated by using several doses of noradrenaline, and the resulting dose-response curves are shown in Figure 3. Two differences can be observed for the longitudinal fibres in the curve obtained by internal perfusion, in relation to that resulting from external perfusion: (a) a shift to the right resulting in a 32 fold decrease in sensitivity (Table 1) and (b) a decrease of 70% in the maximal effect (Table 1). Considering the circular layer, significant differences on maximal effects were not observed, but a small, though significant, difference was obtained for pD_2 values (Table 1). It is noteworthy that in this case the difference was opposite to that shown for the longitudinal layer, since the circular layer was more sensitive to internally perfused noradrenaline.

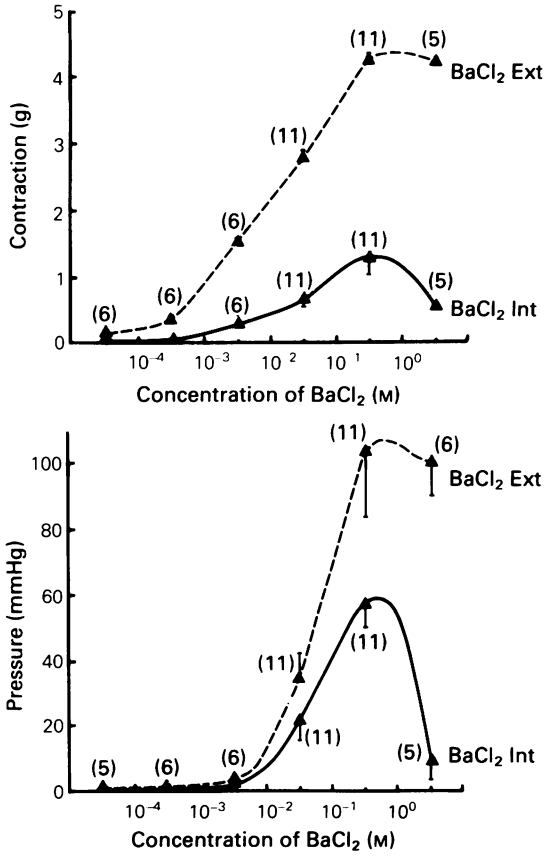

Figure 4 Log dose-response curves for barium chloride, obtained in similar conditions to that for noradrenaline (Figure 3). Differences in maximal contractile effects were also observed. Very little effect on pressure was observed for the largest concentration of BaCl. Number of experiments are in parentheses, vertical bars indicate s.e.mean.

Characteristics of the contractile effects of barium chloride

For the longitudinal external muscle, the dose-response curve resulting from internal perfusion of barium chloride was also lower than that obtained by external perfusion, but no significant differences were found in the position of the curves on the dose axis (Figure 4), and as a consequence, in pD_2 values (Table 1). No significant differences were found for pD_2 values of barium in the circular layer, although a comparison of maximal effects was difficult, because increasing the dose perfused internally elicited very little effect (Figure 4), and caused changes in the osmolarity of the nutrient solution.

Simultaneous internal and external perfusion of noradrenaline

In order to verify whether the lower dose-response curves for drugs applied internally could be due to a desensitization, or to the release of an inhibitory substance through the lumen of the preparation, experiments were done in which drugs were added internally, in order to induce a maximal response, and were followed at this point by a simultaneous external perfusion of the same dose of agonist. Figure 5 shows that an additional contraction could be obtained in these conditions, indicating that not all the receptors had been reached after a maximal contraction through internal perfusion. On the other hand, no additional contraction could be attained by internal perfusion of noradrenaline after obtaining a maximal effect through external perfusion.

Influence of the thickness of the circular layer

The longitudinal muscle layer of the vas deferens is of equal thickness throughout the whole organ length whereas the circular layer is about twice as thick at the prostatic end as at the epididymal end. Therefore, if the circular layer represents a barrier for the internally applied drug to reach the longitudinal muscle layer, one would expect this barrier to be more effective at the prostatic end. Figure 6 represents the effects of maximal doses of noradrenaline and barium chloride, in the prostatic and epididymal halves of the vas deferens. These drugs, when perfused internally through the prostatic end, induced maximal effects which were only about 20% of the maximum induced

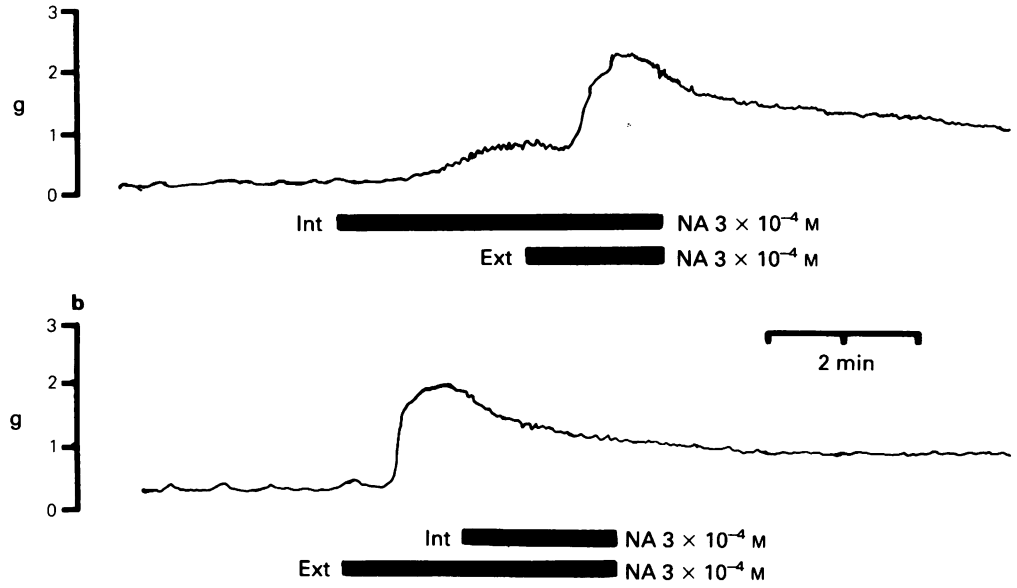

Figure 5 (a) Typical experiment, showing the tension (g) elicited by a maximal dose of noradrenaline (NA) added to the internal fluid (Int) until equilibrium was reached, followed by a simultaneous perfusion with external (Ext) noradrenaline. Note that an additional contraction was recorded after external perfusion. (b) Effect of the same dose of NA, except that external drug perfusion was initiated before internal. In this case an additional contraction was not observed. Horizontal bars indicate the period during which NA was present in the perfusing solutions.

by external perfusion. On the other hand, no differences in maximum effects were observed for the epididymal end, after external and internal perfusion of noradrenaline, and only a small difference was found for barium chloride (Figure 6).

Influence of external phenoxybenzamine on the effect of internal and external noradrenaline

Phenoxybenzamine, an irreversible competitive antagonist, was perfused externally followed by exter-

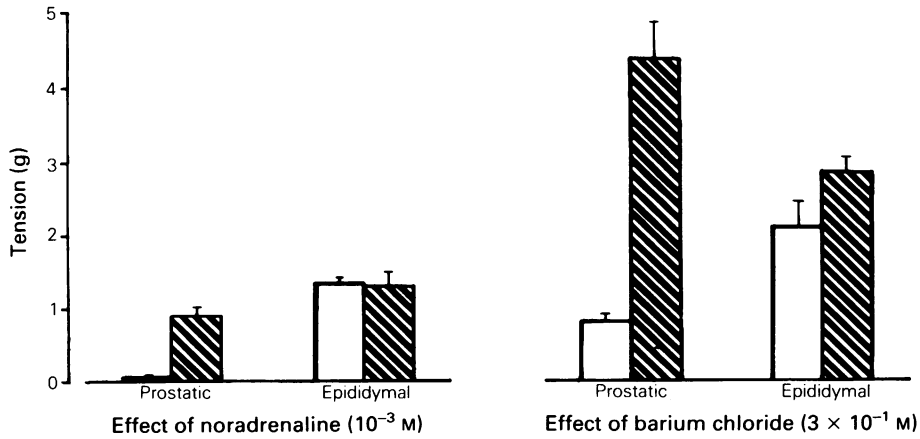

Figure 6 The effect of maximal doses of noradrenaline or of barium chloride added to the internal (open columns) or external (hatched columns) perfusion fluids, on the prostatic or epididymal halves of the vas deferens. Note the large differences on maximum effects obtained on the prostatic part but no significant differences for noradrenaline on the epididymal segment. Each column represents 6 experiments. Vertical bars indicate s.e.mean.

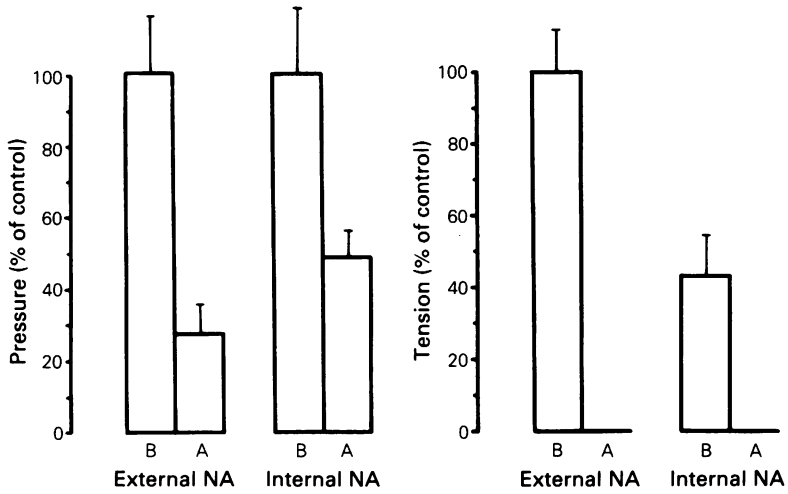

Figure 7 The maximal effects of noradrenaline (10^{-3} M) before (B) and after (A) external perfusion with the irreversible competitive blocker phenoxybenzamine (10^{-5} M, for 2.5 to 4 min). Results are presented as percentages of the effect attained by external NA before phenoxybenzamine. Note that for the longitudinal layer a complete blockade of NA receptors was attained whether the agonist was added internally or externally. Contractions of the circular layer, indicated by increased pressure after NA, persisted, although reduced to less than 50% of the previous control values. Each column represents 8 experiments. Vertical bars indicate s.e.mean.

nal or internal perfusion of noradrenaline. Figure 7 shows that a dose of 10^{-5} M of this antagonist, for up to 4 min, induced a complete blockade of the effect of noradrenaline on the longitudinal layer, although a contraction could still be elicited in the circular layer. This shows that the contractions of the latter layer are not a consequence of a contraction propagated from the longitudinal layer. It must be emphasized also that the inhibition of the contractility of the longitudinal layer was observed whichever the route of noradrenaline perfusion, although phenoxybenzamine had been perfused only externally. This indicates that the action of phenoxybenzamine was not limited to the external surface of the muscle layer, otherwise noradrenaline would have induced a contraction of that layer when added internally.

Attempt to detect inward or outward diffusion of drug

[^{3}H]-noradrenaline was perfused externally in order to verify whether the drug could be detected in the internal perfusion fluid. After 5 min perfusion no detectable amounts of radioactivity could be found. Considering the sensitivity of the method, it may be concluded that if the drug was present in the internal fluid, its concentration was at least one thousand times lower than that in the internal fluid. Experiments were also performed by perfusing the radioactive substance internally and attempting to detect it externally, with similar negative results.

In another series of experiments non-radioactive noradrenaline or barium chloride were perfused internally while the external perfusion fluid was continuously dropped over a second vas deferens preparation, without inducing effects. Dropping the internal perfusion fluid during external drug perfusion induced similar negative results. These experiments, besides showing that the amount of drug crossing the organ wall is negligible in relation to its concentration in the perfusion solution, also demonstrate that our results are not due to drug leakage caused by technical failure.

Discussion

If the effect of a drug on the external longitudinal muscle of the rat vas deferens is expected to follow the simplest postulates of receptor theory (Clark, 1933; Ariens, 1964), we would have obtained superimposed dose-response curves, whichever the route of perfusion. This was not the case for the present experiments: dose-response curves for noradrenaline, when obtained by external perfusion, had a larger pD_2 value (indicating a shift of 1.5 log units to the left), and were about three times higher than when obtained by internal perfusion. Similar differences were found for maximum effects of barium chloride, but not for pD_2 values. Perfusion-related differences were also found on the circular layer to a lesser extent; however, this layer was more sensitive to drugs applied by internal perfusion. Most of the discussion will concern the results obtained in the longitudinal layer, assuming

that the differences were less marked for the circular layer because it is more equidistant than the longitudinal layer in relation to the external and internal surfaces of the organ wall.

From the standpoint of receptor theory, the action of a drug can be divided in three stages: passage from the fluid bathing the tissue to the receptor region within the tissue (i.e. entrance in the biophase and interaction with removal mechanisms in that compartment); interaction with the receptor; and response of the tissue to receptor occupation by drug (Ariens, 1964). Therefore, if the shape of a dose-response curve is changed by experimental conditions, this change ought to be due to a variation in at least one of these three stages. In the present case the entrance of drugs into the receptor compartment is obviously different when comparing external and internal perfusion. Therefore, except where otherwise stated, we shall discuss whether the differences observed in dose-response curves are compatible with differences in this first stage of drug action.

Differences in position of the dose-response curves

Based on theoretical analysis (Furchgott, 1972) and on previous studies with noradrenaline in rat vas deferens we know that noradrenaline, when entering the biophase, has its concentration greatly reduced by neuronal uptake (Jurkiewicz & Jurkiewicz, 1976) and is almost unaffected by extra-neuronal uptake (Langeloh & Jurkiewicz, 1982). As a consequence, the corresponding dose-response curve is located to the right in relation to the curve which would be obtained if neuronal uptake were absent, or blocked (Jurkiewicz & Jurkiewicz, 1976). Therefore, the difference of about 30 times in the sensitivity of the longitudinal muscle to noradrenaline according to the route of application, can be explained on the basis of this possibility: when the drug is added externally it immediately reaches this muscle layer, but when added internally it has to cross the richly innervated circular layer, in which the changes of drug molecules being taken up by nerve terminals are larger. As a consequence larger doses of noradrenaline were needed when the drug was added through the lumen. The possibility of an involvement of uptake mechanisms is strengthened by two additional results: (a) no significant differences were found for pD_2 values for barium chloride, which is not influenced by neuronal uptake; and (b) the internal circular layer was slightly more sensitive to noradrenaline added through the lumen, since this layer is most easily reached by this route. If the differences were due, for instance, to the release of an inhibitory substance from the mucosa then the circular layer should also be less sensitive to internal noradrenaline. Differences in the magnitude of contractile responses after external and internal perfusion have been observed by other investigators, mainly in vascular preparations, and were also mostly attributed to the presence of an active neuronal uptake (De la Lande *et al.*, 1967; Steinsland *et al.*, 1973). However, a complete understanding of the role played by neuronal uptake in the present case will only be obtained after the use of uptake blockers or of denervated preparations.

Differences in the maximum of the dose-response curves

Unlike the differences in pD_2 values, the variations observed in the heights of the dose-response curves cannot be explained by the presence of neuronal uptake: the role of this mechanism is to reduce the concentration of drug in the vicinity of receptors and therefore its influence can always be overcome by increasing the dose of agonist in the organ bath: consequently, the same maximal effect is expected to occur, although with larger doses than those needed for a maximal effect in the absence of the uptake mechanism. However, the situation can be proposed in which drugs are taken up to such a large extent that very little remains free (Paton & Rang, 1965; Rang, 1966; Thron & Waud, 1968). This possibility, which was analysed by Kenakin (1980), on the basis of a theoretical model advanced by Green (1976), would lead to a decrease of the maximum effect of agonists.

At least two other phenomena, not considered in Figure 1, are candidates to explain the lower effects of drugs added internally: (1) a drug-induced release of an inhibitory substance from the mucosa, (Furchgott & Zawadski, 1980), leading to a non-competitive-like antagonism, and (2) the development of desensitization simultaneously with the slow diffusion of drug across the organ wall. Both types of phenomena would interfere with the last stage of drug action, reducing non-specifically the response of the tissue to receptor occupation; as a consequence, this interference would be unsurmountable, i.e. it would not be overcome by increasing the dose of agonist (Ariens, 1964). However, Figure 5 showed that this is not the case, since an additional contraction was attained by adding an external dose simultaneously to the same maximal internal dose. Therefore both possibilities can be discarded as causative of the lower dose-response curve for internally perfused drugs. On the other hand, Figure 5 clearly shows that the cause for this lower effect was the fact that not all the receptors could be reached when the drugs were applied through this route; in fact, the unoccupied receptors could be reached by adding the drug through external perfusion.

If the drugs are supposed to follow the paths illustrated in Figure 1, and if this passage obeys simple diffusion laws, then all the receptors should be reached whichever the route of perfusion. Therefore, if it is

assumed that not all the receptors are reached by internal perfusion, we have to suppose that, in the present case, diffusion has a more complex pattern than that expected from the biophase model.

At least two possibilities for such a mechanism can be considered: (a) the first hypothesis assumes that diffusion is a very slow process, when compared to drug-induced responses and, therefore, from an operational standpoint it may be considered as meaningless or absent. This was proposed by Venter (1981) who showed that isoprenaline requires more than 10 min to approach an equilibrium concentration through a 1 mm-diameter papillary muscle, whereas the peak inotropic response is achieved within a very short time, when the majority of the drug molecules are still at the muscle surface. He therefore suggested that drug-receptor interaction occurs at the receptors located at superficial cells (named 'initiator' cells) and are propagated through the muscle by 'propagator' cells. If our results are analysed in the framework of this hypothesis, it might be assumed that drugs added externally interact with the superficial cells of the longitudinal layer, and that contraction is propagated throughout the whole smooth muscle, including the circular layer; on the other hand, when drugs are perfused through the lumen, the contraction is propagated from the internal surface of the circular layer. In other words, contraction would be initiated by interactions with two independent sub-populations of receptors, which would explain the different dose-response curves shown in Figure 3 and the additive effect in Figure 5a. However this hypothesis does not explain the experiments shown in Figure 7, in which external noradrenaline was able to contract the circular layer, without contracting the longitudinal muscle, though passing through it. Another drawback for this hypothesis is that one would not expect externally added phenoxybenzamine to block or even reduce the effect of internal noradrenaline (Figure 7). Therefore this hypothesis does not fully explain our results. (b) The second hypothesis has not been previously analysed by other authors, to our knowledge, and considers the possibility that drug diffusion varies during the performance of a dose-response curve. It is based on the fact that the circular muscle acts as a dynamic layer, since the tissue will contract as soon as the first drug molecules reach the fibres. The degree of contraction will increase in proportion to the concentration of drug molecules crossing that layer. This contraction might cause a dose-dependent change of the permeability of smooth muscle tissue to agonists, contrary to the expectations of the usual models for drug-diffusion which consider drug diffusion to occur through static barriers. A lower maximum effect would be expected if the highest doses acted to decrease the permeability of the muscle to diffusion. If this is the case one would expect antagonists, which do not cause muscle contraction, to diffuse differently from agonists. However this possibility needs further experimentation.

In conclusion, it can be assumed that the perfusion-related differences in dose-response curves for agonists in rat vas deferens are due to two phenomena: (a) the presence of neuronal uptake, which is most effective when the drug is applied by internal perfusion, causing differences in the position of the curves of noradrenaline on the dose axis but not on maximum effects, and (b) a limitation of the access of internally added noradrenaline or barium to part of the respective population of receptors, leading to decreased heights of the respective curves. The causes for the latter phenomenon remain to be determined.

References

ANSTEY, M. (1971). The circular muscle of the vas deferens of the guinea-pig. *J. Physiol.*, **222**, 81P–82P.

ANSTEY, M.D., BIRMINGHAM, A.T. & TOMLINSON, D.E. (1974). The noradrenaline concentration and the cholinesterase activity of the separated longitudinal and circular layers of muscle of the guinea-pig vas deferens. *Br. J. Pharmac.*, **50**, 454P–455P.

ANSTEY, M.D. & BIRMINGHAM, A.T. (1978). The response of the circular muscle layer of the guinea-pig isolated vas deferens to transmural electrical stimulation. *Br. J. Pharmac.*, **63**, 617–625.

ANSTEY, M.D. & BIRMINGHAM, A.T. (1980). The isolated circular muscle layer of the vas deferens of the guinea-pig. *Br. J. Pharmac.*, **69**, 233–239.

ARIENS, E.J. (1964). *Molecular Pharmacology*. Vol. 1. New York: Academic Press.

BUSATTO, P.A. & JURKIEWICZ, A. (1976). Efeito de drogas aplicadas internamente e externamente no ducto deferente de rato. *Ciencia e Cultura* (São Paulo), **28**, 549 (Abs).

BUSATTO, P.A. AND JURKIEWICZ, A. (1977). Biofase com barreiras variáveis: análise teórica e experimental. *Ciencia e Cultura* (São Paulo), **29**, 600 (Abs).

CLARK, E.J. (1933). *The Mode of Action of Drugs on Cells*, London: Arnold.

DE LA LANDE, I.S., FREWIN, D.B., AND WATERSON, J.G. (1967). Influence of sympathetic innervation on vascular sensitivity to noradrenaline. *Br. J. Pharmac.*, **31**, 82–93.

FURCHGOTT, R.F. (1955). The pharmacology of vascular smooth muscle. *Pharmac. Rev.*, **7**, 183–265.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Handbook of Experimental Pharmacology*, Vol. 33, pp. 283–335. ed. Blaschko, H. & Muscholl, E. New York: Springer-Verlag.

FURCHGOTT, R.F. & ZAWADSKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

GREEN, A.L. (1976). The kinetics of enzyme action and

inhibition in intact tissues and tissue slices, with special reference to cholinesterase. *J. Pharm. Pharmac.*, **28,** 265–274.

JACKSON, A.R. & TOMLINSON, A.R. (1978). An analysis of the response of the perfused vas deferens of the rat to field stimulation. *Br. J. Pharmac.*, **64,** 467–468P.

JURKIEWICZ, A., FURCHGOTT, R.F. & JURKIEWICZ, N.H. (1973). Kinetics of recovery from propranolol-blockade in guinea-pig trachea. *Proceedings of the 2nd Meeting on Adrenergic Mechanisms*, Oporto, p. 23.

JURKIEWICZ, A. & JURKIEWICZ, N.H. (1976). Dual effect of α-adrenergic blockers in rat vas deferens. *Br. J. Pharmac.*, **56,** 169–178.

JURKIEWICZ, A., BUSATTO, P.A., LANGELOH, A., JURKIEWICZ, N.H. & HIRATA, H. (1978). An analysis of the biophase model by means of internal and external perfusion of the rat vas deferens. *Abstracts of the 7th International Congress of Pharmacology*, Paris, p. 82.

KENAKIN, T.P. (1980). On the importance of agonist concentration-gradients within isolated tissues. Increased maximal responses of rat vasa deferentia to (−)-noradrenaline after blockade of neuronal uptake. *J. Pharm. Pharmac.*, **32,** 833–838.

LANGELOH, A. & JURKIEWICZ, A. (1982). Analysis of the effects of dopamine and noradrenaline in relation to the proposed postsynaptic dopamine receptor in rat vas deferens. *Naunyn-Schmiedebergs Arch. Pharmac.*, **318,** 202–209.

MARTINS, T. & VALLE, J.R. (1939). Endocrine control of the motility of the male, accessory genital organs. *Endocrinol.*, **25,** 80–85.

PATON, W.D.M. & RANG, H.P. (1965). The uptake of atropine and related drugs by intestinal smooth muscle of the guinea-pig in relation to acetylcholine receptor. *Proc. R. Soc., B.*, **163,** 1–44.

PATON, W.D.M. & WAUD, D.R. (1964). A quantitative investigation of the relationship between rate of access of a drug to receptor and the rate of onset or offset of action. *Arch. exp. Path. Pharmak.*, **248,** 124–143.

RANG, H.P. (1966). The kinetics of action of acetylcholine antagonists on smooth muscle. *Proc. R. Soc., B*, **164,** 488–510.

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

STEINSLAND, O.S., FURCHGOTT, R.F. & KIRPEKAR, S.M. (1973). Biphasic vasoconstriction of the rabbit ear artery. *Circulation Res.*, **32,** 49–58.

THRON, C.D. & WAUD, D.R. (1968). The rate of action of atropine. *J. Pharmac. exp. Ther.*, **160,** 91–105.

VENTER, C.J. (1981). β-adrenoceptors, adenylate cyclase, and the adrenergic control of cardiac contractility. *Adrenoceptors and Catecholamine Action.* ed. Kunos, G. pp. 213–245. New York: John Wiley & Sons.

(Received August 9, 1983.
Revised November 13, 1984.
Accepted May 2, 1985.)