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Effect of Alpha-1 and Beta Agonists on Contraction of Bovine Retinal Resistance Arteries In Vitro

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Contractile responses of bovine retinal arteries (BRA) (diameter: 179 ± 9 μm, n = 25) to high K^+, circumferential stretch and adrenergic stimulation were studied in vitro. BRA could be activated by rapid circumferential stretch. Under resting conditions, phenylephrine consistently activated BRA at the highest dose of the drug used (10^{-5} M). During K^+- and stretch-induced activation, significant contractile responses to phenylephrine appeared at lower doses (respectively, 3.10^{-8} and 10^{-6} M). Isoproterenol did not relax K^+- and stretch-induced contractions. Therefore, (1) BRA probably can autoregulate through a myogenic mechanism on the basis of stretch; (2) during alpha$_1$ adrenergic stimulation, myogenic autoregulatory responses probably increase; (3) contractile responses to alpha$_1$ adrenergic stimulation are masked under resting conditions; and (4) BRA may not possess functional beta adrenergic receptors. Invest Ophthalmol Vis Sci 30:44–50, 1989

The effects on retinal blood flow of various alpha and beta adrenergic agonists and antagonists applied topically have been studied.[1–5] However, precise knowledge of presence and type of adrenergic receptors in the retinal vascular bed is still lacking. Recently it was shown that bovine retinal vessels have alpha adrenergic binding sites.[6] A technique to measure isometric contractions of microvessels in vitro[7] enabled us to investigate the contractile responses to alpha$_1$ and beta adrenergic drugs of the main retinal arteries, ie, the arterial segments located between the optic disc and the first intraretinal arterial branching. Bovine retinal arteries were used, as these vessels can be isolated with minimal trauma and as both cow and man have the same type of holangiotic (or euangiotic) retinal vascularization.[8]

Materials and Methods

Biological Material and Organ Bath

Twenty-five bovine retinal arteries (BRA) were used in this study. Bovine eyes were obtained at the local abattoir, at latest 30 min after death. The eyes were transported to the laboratory in physiological salt solution (PSS) at 4°C, containing (mM): NaCl 118; KCl 4.7; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 24; $CaCl_2$ 2.5; and glucose 4.5 (pH 7.35–7.4). The anterior segment and vitreous body were removed. Under a dissecting microscope, a segment of the most prominent BRA, located just before the first intraretinal branching, was carefully dissected free of the accompanying vein and surrounding retinal tissue. Two stainless steel wires of 40 μm diameter were inserted through the arterial lumen. Subsequently, the whole preparation (BRA segment with wires) was excised (longitudinal length of the BRA segment: 1.89 ± 0.04 mm) and gently transferred to an organ bath filled with 5 ml PSS. The two wires were connected to two specimen holders (Fig. 1). One of these specimen holders was connected to a three-dimensional micromanipulator and the other to the lever of an electromagnetic force-length transducer (see "Transducer and Recording System"). The PSS was maintained at 37°C and bubbled with a gas mixture of 95% O_2 and 5% CO_2. The PSS in the organ bath could be rapidly replaced by prewarmed and oxygenated PSS from a reservoir (Fig. 1), while the preparation remained immersed in fluid and causing minimal mechanical disturbances.

Activation of BRA

BRA showed no spontaneous contractile activity in PSS. Therefore, contractions were induced by an activating solution obtained from a second reservoir (Fig. 1), containing 125 mM K^+ (K^+-PSS, equimolar replacement of Na^+) and activating the smooth muscle cells by depolarization. A second way to activate

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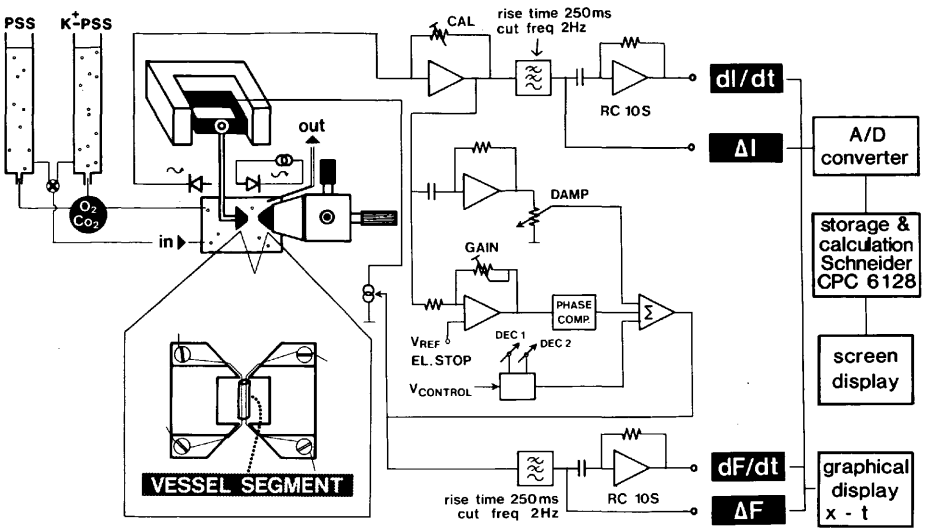

Fig. 1. Schematic representation of the organ bath, force-length transducer and recording system. Bottom part of the figure shows enlarged top view of the two specimen holders with mounted vessel segment. The right specimen holder is connected to a three-dimensional micromanipulator and the left holder to the lever of the transducer. Right part shows electronic circuitry of the transducer with output signals, left part the thermostatted oxygenated PSS reservoirs with relaxing solution (normal PSS) and activating solution (K^+-PSS) connected with a three-way valve to the organ bath.

BRA was by rapid circumferential stretch ("Transducer and Recording System").

Transducer and Recording System

Isometric contractions were studied: the activated BRA were not allowed to shorten circumferentially, but developed force in stead (units: 1 mN = 9.8 mg). Constant circumference and force measurements were accomplished by the specific design of the electromagnetic force-length transducer (Fig. 1).

The position of the lever of the transducer, which determined the distance between the two intraluminal wires and the circumference of the BRA, could be chosen by the investigator. Then, this position was maintained by a length-sensing electronic feedback circuit. This circuit was based on the lever itself which acted as a shutter between an infrared light-emitting diode and a light-sensitive diode.

Force measurements were accomplished by the attachment of the lever of the transducer to a coil which rotated freely in the field of a magnet (Fig. 1). When the BRA developed force, the lever moved a very small amount. This movement triggered the length-sensing feedback circuit which then kept the lever at the chosen position by appropriate increases of the current through the coil, which counteracted further movement of the lever. The current was generated by a current source calibrated in mN.

Finally, abrupt changes (20 msec) between two lever positions (chosen by the investigator) could be achieved by switching manually between two length-sensing feedback circuits which both controlled the position of the lever. An abrupt lever position change resulted in a rapid change of circumference of the BRA.

Force and length signals were recorded on a 6-channel Watanabe WTR 331 pen recorder and in selected cases captured with a microcomputer data acquisition system (Schneider CPC 6128 and data acquisition system module made at the departmental workshop). All parameters were displayed on screen as a function of time, and stored on floppy discs. Figures were either retraced from chart records or plotted on a Hitachi 672 Graph-Plotter from the data stored on floppy disc.

Experimental Protocols

After mounting, ie, connecting the two intraluminal wires to the specimen holders, the BRA were allowed to stabilize for at least 1 hr at a small circumference. Passive force, ie, the force needed to stretch the BRA to this circumference, was always smaller

than 0.4 mN. Subsequently, l_{max}, ie, the circumference at which the preparation developed maximal active force, was determined. This was done by slowly increasing the circumference of the BRA in 10–30 μm steps and determining at each circumference the active force developed during K^+ activation, until the circumference was reached at which no further increase of active force development was noted. This circumference was the l_{max} for the BRA under study. All experiments were performed at l_{max}. BRA internal circumference at l_{max} was 575 ± 26 μm (internal diameter: 179 ± 9 μm, range 97 to 248 μm). Passive force at l_{max}, ie, the force needed to stretch the BRA to l_{max}, was 0.25 ± 0.02 mN/mm^2. The ratio of passive to total force (active developed force + passive force) during K^+ activation was determined and only BRA with ratios smaller than 0.15 were used, ensuring that experiments were done only on BRA which did not suffer extensively from the isolation procedure or from in vitro conditions.

Pharmacological interventions were performed on resting (normal PSS) or on activated (K^+-PSS) BRA. With a first procedure the drug under study was added to stabilized, K^+-activated BRA and the response was expressed with reference to the force prior to drug application. With a second procedure, resting BRA were equilibrated with the drug under study, after which active responses to stretch were obtained in the presence of the drug. These responses were compared to control contractions prior to drug application and after wash.

Statistics and Calculations

Force was normalized by the vessel wall surface, ie, the internal circumference at l_{max} and the longitudinal length of the BRA segment. Calculation of the internal circumference was performed according to Hogestatt et al.[9] Longitudinal segment length was measured with the aid of a microscope with a calibrated eye piece.

All data were expressed as means ± standard error and were statistically analyzed using paired student t-test, taking $P \leq 0.05$ as the limit of significance. For each experimental protocol, the number of BRA reported is also the number of animals used. The tracings in *Results* illustrate findings obtained in multiple experiments.

Drugs Used

All drugs were disolved in distilled water and added as 5 μl volumes to the organ bath. Drugs were obtained from the following sources: phenylephrine, Winthrop (New York, NY); phentolamine, Ciba-Geigy (Ardsley, NY); isoproterenol and papaverine, Sigma (St. Louis, MO).

Abbreviations used:

BRA:	bovine retinal arteries.
PSS:	physiological salt solution.
K^+-PSS:	physiological salt solution with Na^+ replaced by K^+ on an equimolar basis; K^+ = 125 mM.
l_{max}:	BRA circumference at which the preparation developed maximal active force during K^+-induced activation.

Results

Activation of Bovine Retinal Arteries

K^+-induced contractions: K^+-induced contractions had a high degree of reproducibility and were characterised by a rapidly developing peak (phasic part). Time to peak force was 42 ± 7 seconds and active force was 2.01 ± 0.15 mN/mm^2 (n = 16). Subsequently, active force stabilized at a plateau (tonic part) which was 73 ± 7% of phasic peak force. This tonic part was maintained as long as K^+-PSS was present (at least 10 min) and was immediately relaxed in normal PSS (Fig. 2).

Stretch-induced contractions: After rapid stretch of bovine retinal arteries (BRA) from 0.67 ± 0.04 l_{max} to l_{max} (n = 11), force transients were observed (Fig. 3). An immediate large force peak was followed by a slower and smaller force transient. Active force during the slower transient was 0.28 ± 0.04 mN/mm^2 or 12 ± 2% of K^+-induced peak force. Prior addition of 10^{-6} M papaverine (n = 4) totally abolished active force development (Fig. 4A).

The first stretch-induced contraction had a depressant effect on the subsequent contraction (Fig. 3, second part). To obtain reproducible contractile responses to stretch, each stretch-induced contraction had to be preceded by at least two K^+-induced contractions at regular time intervals.

Effect of Phenylephrine

Response during resting conditions: Cumulative doses of phenylephrine (10^{-8} to 10^{-5} M) were added to BRA under resting conditions (n = 8). BRA did not respond to phenylephrine up to 3.10^{-7} M. Six BRA preparations showed small contractions when exposed to larger concentrations of the drug (Figs. 5, 6). These contractions were highly transient: after 36 seconds, the contractile response to 10^{-6} M phenylephrine had declined to 17 ± 6% of the initial response. Contractile responses to phenylephrine could be elicited repeatedly, but force development showed considerable variability over the time course of an experiment (Fig. 7). Two out of eight BRA showed

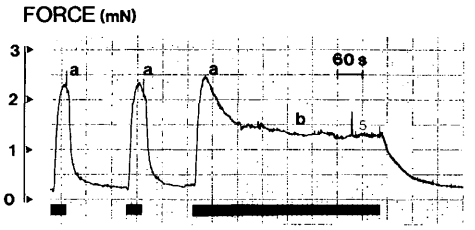

Fig. 2. K^+-induced contractions. Black bars indicate the presence of K^+ 125 mM. (a): phasic and (b): tonic part of the K^+-induced contraction.

small and rapid spontaneous contractions in the presence of 10^{-5} M phenylephrine (see also Fig. 5).

Response during K^+-induced activation: Stabilized K^+-activated BRA (n = 8) were exposed to increasing concentrations of phenylephrine (10^{-8} to 10^{-5} M) (Fig. 8). Active force increased concentration dependently to 228 ± 24% of K^+-induced tonic force at 10^{-5} M (Fig. 6). At this concentration, active force was 47 ± 8% larger still than at K^+-induced peak force. The responses to phenylephrine were now more sustained: after 36 seconds the contractile response to 10^{-6} M phenylephrine was still 79 ± 7% of the initial response.

Response during stretch-induced activation: Stretch was applied to BRA (n = 8) as soon as the transient response to phenylephrine under resting conditions, had subsided. Phenylephrine potentiated stretch-induced contractions dose-dependently (Fig. 4). Active

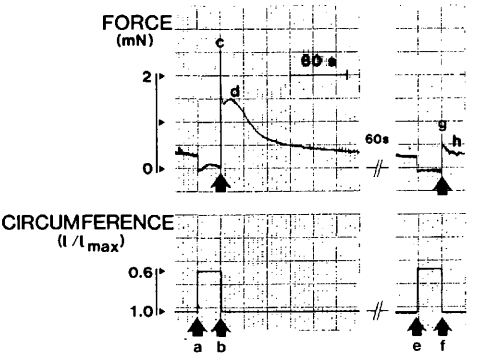

Fig. 3. Representative example of a stretch-induced contraction. The resting BRA was allowed to stabilize at a small circumference during 25 seconds (a) and was then rapidly stretched to l_{max} (b), which induced two force transients: an immediate force peak (c) and a slower force peak (d) which relaxed spontaneously. Immediate repetition of this procedure (e, f) showed poor reproducibility (g, h).

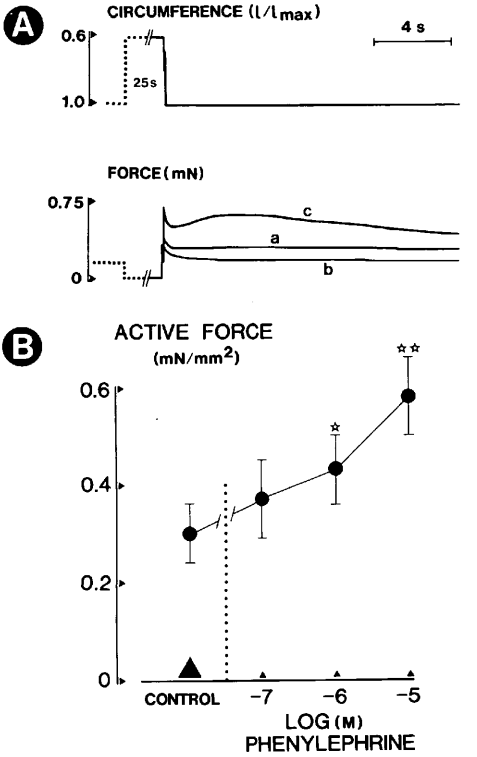

Fig. 4. Response to phenylephrine or papaverine during stretch-induced activation. (A) Representative example. Stretch from 0.61_{max} to 1_{max} was applied during control conditions (a) and in the presence of papaverine 10^{-6} M (b) or phenylephrine 10^{-5} M (c). During papaverine, force after stretch equaled passive force at 1_{max}; during control conditions BRA developed active force. During phenylephrine active developed force was enhanced. (B) Mean active developed force during stretch-induced activation in the absence and in the presence of different doses of phenylephrine (n = 8; *: $P < 0.05$; **: $P < 0.01$).

force after stretch in the presence of 10^{-5} M phenylephrine was 219 ± 34% of control.

Effect of Isoproterenol

Adding isoproterenol (10^{-6} M) to BRA (n = 5) under resting conditions did not induce contractile responses. In stabilized K^+-activated BRA (n = 6), increasing concentrations of isoproterenol (10^{-8} to 10^{-5} M) had no effect on tonic force up to 10^{-6} M (Fig. 9). Higher doses of isoproterenol (10^{-5} M) slightly enhanced active developed force to 146 ± 18% of K^+-induced tonic force. Similarly, isopro-

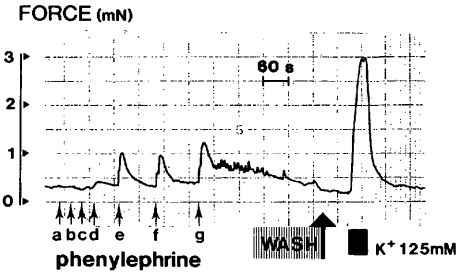

Fig. 5. Representative example of the dose response to phenylephrine during resting conditions. Cumulative doses of phenylephrine were added (M): (a): 10^{-8}; (b): 3.10^{-8}; (c): 10^{-7}; (d): 3.10^{-7}; (e): 10^{-6}; (f): 3.10^{-6}; (g): 10^{-5}. Only high doses of phenylephrine induced contractions, which spontaneously relaxed and which were small compared to the K^+-induced contraction.

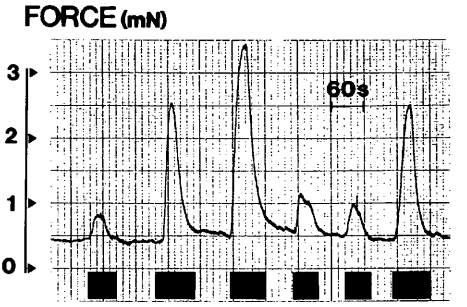

Fig. 7. Representative example of the response to repeated exposure to phenylephrine during resting conditions. Black bars indicate the presence of phenylephrine 10^{-6} M. Contractions were transient and variable.

terenol did not relax stretch-induced contractions (n = 2; active force after stretch in the presence of 10^{-6} M isoproterenol: 114% and 125% of control).

Discussion

A technique developed recently to measure force development of microvessels in vitro[7] was used to investigate activation of bovine retinal arteries (BRA) and to analyze the contractile responses of BRA to alpha$_1$ and beta adrenergic stimulation.

Stretch-induced activation was demonstrated in BRA, ie, BRA developed force transients after rapid circumferential stretch. The immediate force transient was due to stretch of the passive elastic elements in the arterial wall.[10] The subsequent slower transient, however, was caused by active force development of the smooth muscle cells, as it could be inhibited pharmacologically by papaverine, a smooth muscle relaxant. Circumferential stretch occurs in vivo after sudden elevation of perfusion pressure, after which resistance vessels narrow their diameter to keep flow constant. Therefore, vascular segments which show stretch-induced activation in vitro, are

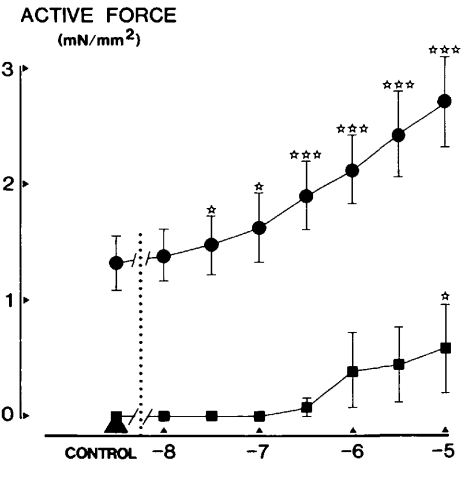

Fig. 6. Mean active developed force during resting conditions (■) and during K^+-induced activation (●) at each concentration of phenylephrine (n = 8; *: $P < 0.05$, ***: $P < 0.001$). Control for K^+-activated BRA was tonic force during K^+ activation.

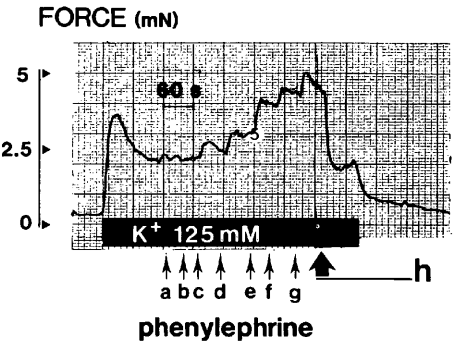

Fig. 8. Representative example of the response to phenylephrine during K^+-induced activation. Cumulative doses of phenylephrine were added (a → g, see legend Fig. 5) to stabilized K^+-activated BRA. Adding phentolamine 10^{-5} M (h) after stabilization in the presence of phenylephrine 10^{-5} M, reversed the effect of phenylephrine.

thought to be able to autoregulate in response to elevated perfusion pressure by a myogenic mechanism.[11,12] Conversely, one could conceive that at least part of the autoregulatory behaviour of retinal blood flow in response to reduced perfusion pressure[13,14] can be explained by the inverse of this myogenic mechanism. According to Bevan,[11] stretch-dependent tone is the most important determinant of vascular resistance.

BRA under resting conditions reacted poorly to alpha$_1$ adrenergic stimulation, ie, they merely developed small, transient and variable contractions at high doses of phenylephrine. This cannot be attributed to the use of the in vitro technique, as vessels of similar size in other species and vascular beds, studied with a similar technique, responded to alpha adrenergic stimulation by sustained, reproducible contractions comparable to K^+-induced contractions.[15-17]

As BRA in vivo probably possess a basal tone and are therefore in an activated state, phenylephrine was added to activated BRA. In K^+-activated BRA, phenylephrine persistently enhanced active developed force, even at low concentrations. At high concentrations, BRA developed more force still than during the phasic part of the K^+-induced contraction. Phenylephrine also enhanced active force development during stretch-induced activation, indicating that the vasoconstrictor response to elevated perfusion pressure is exaggerated during alpha$_1$ adrenergic stimulation. Conversely, vasodilation in response to reduced perfusion pressure would be largely inhibited during alpha$_1$ adrenergic stimulation.

The beta adrenergic agonist isoproterenol produced no effect in either resting or K^+-activated BRA up to 10^{-6} M, indicating that no functional beta adrenergic receptors were present. If human retinal arteries resemble BRA in this respect, beta adrenolytic drugs (as applied topically during antiglaucomatous therapy) do not affect vascular smooth muscle contraction at the level of the main retinal arteries through beta adrenergic binding capacities. However, the possibility of a nonspecific action of these drugs on retinal vascular smooth muscle has yet to be excluded.

In conclusion, we have shown that bovine retinal arteries in an activated state are sensitive to the vasoconstrictor action of alpha$_1$ adrenergic agonists. The contractile response to alpha$_1$ adrenergic stimulation is greatly masked during resting conditions. The myogenic vasoconstrictor response to elevated perfusion pressure is exaggerated during alpha$_1$ adrenergic stimulation. As myogenic tone is thought to be a major determinant of vascular tone, alpha$_1$ adrenergic stimulation could increase retinal vascular resistance. Bovine retinal arteries probably do not possess functional beta adrenergic receptors.

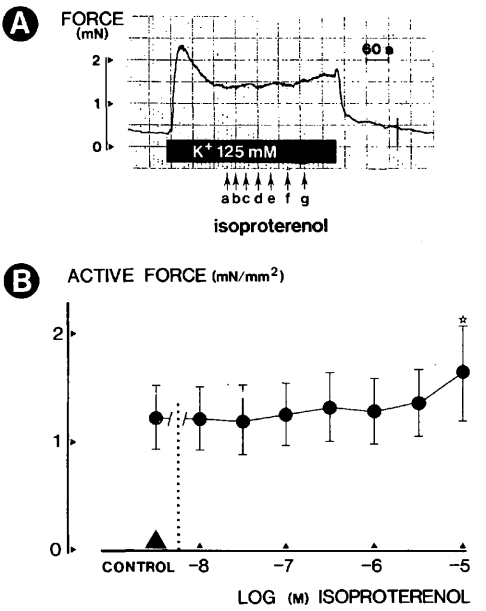

Fig. 9. Response to isoproterenol during K^+-induced activation. (A) Representative example. Cumulative doses of isoproterenol were added (a → g, see legend Fig. 5) to stabilized K^+-activated BRA. (B) Mean active developed force during each concentration of isoproterenol (n = 6; *: $P < 0.05$). Control was tonic force during K^+ activation.

Key words: microvessels, K^+ contractions, myogenic autoregulation, phenylephrine, isoproterenol

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