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High concentrations of KCl release noradrenaline from noradrenergic neurons in the rat anococcygeus muscle

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Abstract

The aim of the present study was to investigate the effects of high concentrations of KCl in releasing noradrenaline from sympathetic nerves and its actions on postsynaptic α-adrenoceptors. We measured the isotonic contractions induced by KCl in the isolated rat anococcygeus muscle under different experimental conditions. The contractile responses induced by KCl were inhibited by α-adrenoceptor antagonists in 2.5 mM Ca^{2+} solution. Prazosin reduced the maximum effect from 100 to 53.9 ± 10.2% ($P<0.05$) while the pD_2 values were not changed. The contractile responses induced by KCl were abolished by prazosin in Ca^{2+}-free solution ($P<0.05$). Treatment of the rats with reserpine reduced the maximum effect induced by KCl as compared to the contractile responses induced by acetylcholine from 339.5 ± 157.8 to 167.3 ± 65.5% ($P<0.05$), and increased the pD_2 from 1.57 ± 0.01 to 1.65 ± 0.006 ($P<0.05$), but abolished the inhibitory effect of prazosin ($P<0.05$). In contrast, L-NAME increased the contractile responses induced by 120 mM KCl by 6.2 ± 2.3% ($P<0.05$), indicating that KCl could stimulate the neurons that release nitric oxide, an inhibitory component of the contractile response induced by KCl. Our results indicate that high concentrations of KCl induce the release of noradrenaline from noradrenergic neurons, which interacts with α_1-adrenoceptors in smooth muscle cells, producing a contractile response in 2.5 mM Ca^{2+} (100%) and in Ca^{2+}-free solution, part of which is due to a direct effect of KCl on the rat anococcygeus muscle.

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- KCl
- Anococcygeus muscle
- α-Adrenoceptors
- Noradrenergic neurons

Introduction

The rat anococcygeus smooth muscle, first described by Gillespie (1), is a paired smooth muscle with a dense sympathetic innervation which represents most of its total innervation in addition to a variety of innervations including cholinergic, serotoninergic, purinergic, and non-adrenergic, non-cholinergic (NANC) ones. Thus, noradrenaline is the main neurotransmitter in the anococcygeus muscle (2,3) which has become a useful preparation for pharmacological studies of the responses of smooth muscle involving sympathetic innervation (4,5).

The synthesized noradrenaline is taken up and retained by granules in the noradrenergic nerve endings which are depleted of

noradrenaline stores by reserpine in organs and noradrenergic nerves. The release of this neurotransmitter from presynaptic nerve terminals is initiated by an increase in intraterminal Ca^{2+} concentration (6). Depolarization of noradrenergic nerve terminals by potassium chloride (KCl) promotes noradrenaline release in several tissues by increasing the influx of extracellular Ca^{2+} into the nerve fiber (7). As shown by Blaustein (8), the depolarization of the varicosity membrane by high concentrations of KCl opens the voltage-operated Ca^{2+} channels in the noradrenergic innervation and promotes Ca^{2+} influx, causing noradrenaline release from the terminal endings of noradrenergic nerves *in vitro*. The Ca^{2+} current is of slow onset and long duration, and is mediated by voltage-sensitive Ca^{2+} channels in the presynaptic terminals. The sensitivity of Ca^{2+} channels to dihydropyridine blockade in the nerve terminal may depend on specific conditions such as repetitive depolarization (9).

Noradrenaline released from sympathetic nerves binds to α_1-adrenoceptors, which are involved in a variety of important physiological processes. These surface receptors initiate signals in their target cells by increasing the concentration of free cytosolic Ca^{2+}, thereby affecting the contractile state of the cell. The interaction of noradrenaline with α_1-adrenoceptors in smooth muscle cells promotes the activation of phospholipase C, with production of inositol-1,4,5-trisphosphate and diacylglycerol (10). Inositol-1,4,5-trisphosphate plays an important role in the mobilization of intracellular Ca^{2+} stored in the sarcoplasmic reticulum (11,12). On the other hand, diacylglycerol activates protein kinase C, which opens the Ca^{2+} channels in the plasma membrane (13), resulting in extracellular Ca^{2+} influx. There are many different types of channels in the plasma membrane through which extracellular Ca^{2+} might enter the cell, such as the L-type channels, which are voltage-operated. A high extracellular concentration of KCl and a depolarizing electrical stimulus promote Ca^{2+} influx by voltage-operated channels (14). The L-type Ca^{2+} channels require relatively strong depolarization for activation, are slowly inactivated, and are blocked by dihydropyridines like nifedipine. This type of Ca^{2+} channels is mainly present in smooth muscle cells (15).

In rat anococcygeus smooth muscle, the released neurotransmitter is noradrenaline which binds to α_1-adrenoceptors, inducing the contractile response (16). Ca^{2+} channels opened by inositol-1,4,5-trisphosphate and diacylglycerol allow Ca^{2+} influx when neurotransmitters bind to α_1-adrenoceptors (17). Gillespie (1) demonstrated the presence of α_1-adrenoceptors in the rat anococcygeus muscle, that are more effectively blocked by prazosin (an α_1-adrenoceptor antagonist) than phentolamine (an α-adrenoceptor non-selective antagonist) (18). Additionally, Docherty and Starke (19) demonstrated the presence of few α_2-adrenoceptors in rat anococcygeus smooth muscle. A subdivision of α_1-adrenoceptors in the rat anococcygeus muscle has been first suggested on the basis of their relative sensitivity to Sgd 101/75 (4(2-imidazoline-amino)2-methylindazol-chlorhydrate) at concentrations at which noradrenaline has a relatively small effect (20). McGrath (21) later showed the contribution of postjunctional α-adrenoceptors, noradrenergic transmission, and a dense population of α_{1A}-adrenoceptors in the anococcygeus muscle. It is possible to differentiate the subtype of α_1-adrenoceptors by using competitive and selective antagonists such as WB-4101, a selective α_{1A}-adrenoceptor antagonist (22).

The inhibitory NANC neurotransmission is regarded to be solely nitrergic since potent inhibitors of nitric oxide synthase such as N^G-monomethyl-L-arginine (L-NMMA) and N^G-L-nitro-L-arginine (L-NOARG) block relaxation in response to NANC stimulation (23). According to Selemidis and Cocks (24), in rat anococcygeus muscle contracted by

phenylephrine, relaxations to NANC nerves were markedly reduced or abolished by treatment with L-NOARG or the nitric oxide scavenger hemoglobin. Taken together, these data show that NANC innervation can influence the contractile responses induced by KCl when the main sympathetic innervation is damaged.

Our hypothesis is that part of the KCl response is due to depolarization of the noradrenergic nerve endings. Thus, in the present study we investigated the effects of high concentrations of KCl in releasing noradrenaline from sympathetic nerves, and the actions of this neurotransmitter on postsynaptic α-adrenoceptors.

Material and Methods

Male Wistar rats (180-200 g) were killed by cervical dislocation, and the anococcygeus muscle was isolated, removed and suspended in a 10-ml organ chamber containing Krebs solution of the following composition: 118.0 mM NaCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 25.0 mM $NaHCO_3$, and 11.2 mM dextrose, at 37ºC, pH 7.4. For the experiments done in Ca^{2+}-free solution we used Krebs solution without $CaCl_2$, containing 30 µM ethylene glycol-bis (ß-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA). One of the muscle extremities was connected to an isotonic transducer (50630-45, Harward Bioscience, South Natick, MA, USA), and the other was connected to a fixed support in the chamber in order to record the tension on a Harward Bioscience Oscillograph polygraph.

Muscle preparations were set at a resting tension of 0.5 g and allowed to equilibrate for 1 h in Krebs solution. The preparations were first stimulated with acetylcholine (1 µM) until reproducible responses were obtained. After thorough washing, the anococcygeus muscle preparations were stimulated with increasing concentrations of KCl from 4.7 to 120 mM. In the solutions with higher KCl concentrations, the NaCl concentrations were proportionally reduced in order to keep the osmolarity. KCl concentration was increased by changing the solution each time a stable plateau was attained.

In order to investigate the reproducibility of the KCl effect with time, the KCl concentration-response curves in 2.5 mM Ca^{2+} or Ca^{2+}-free EGTA solution were repeated after 20 min, which was the time of incubation used for all the antagonists in the specific protocols.

Effects of α-adrenoceptor antagonists on the contractile responses induced by KCl

Concentration-effect curves for KCl (4.7 to 120 mM) were constructed in Krebs solution and in Ca^{2+}-free solution before and after 20-min incubation with the given antagonists: 1 µM phentolamine (a non-selective α antagonist), 1 µM prazosin (a selective α_1 antagonist), or 10 nM WB-4101 (a selective α_{1A} antagonist).

Effect of reserpine on the contractile responses induced by KCl

Concentration-effect curves for KCl (4.7 to 120 mM) were constructed in Krebs solution and in Ca^{2+}-free solution using preparations from rats treated with reserpine (5 mg/kg, *ip*) 48, 24 and 12 h before decapitation. At the beginning of the experiments, tyramine (1 µM) was added to the bath to test the efficacy of reserpine treatment. The treatment with reserpine was considered to be effective when no contractile response induced by tyramine was observed.

To test the influence of the NANC nerves, concentration-effect curves were constructed in Krebs solution in the absence and in the presence of N^G-nitro-L-arginine methyl ester (L-NAME) in the anococcygeus muscle preparations of rats treated with reserpine as described above. In the groups of experiments comparing the effects of treatment

with reserpine on the contractile responses induced by KCl on preparations isolated from control rats, the responses were calculated in relation to those obtained with 1 μM acetylcholine (100%). In addition, we studied the effect of prazosin (1 μM) on the contractile responses induced by KCl (4.7 to 120 mM) in preparations from rats treated with reserpine.

Effect of nifedipine on the contractile responses induced by KCl and noradrenaline

Concentration-effect curves for KCl (4.7 to 120 mM) or noradrenaline (1 nM to 30 μM) were constructed in Krebs solution before and after 20-min incubation with nifedipine (1 μM).

Statistical analysis

The contractile responses are reported as percent of the maximum contraction induced by 120 mM KCl. All the contractile responses obtained in the second concentration-effect curve were compared to the first induced by KCl (control), except in the experiments using preparations from rats treated with reserpine. In this case, the responses to KCl were compared to those obtained with 1 μM acetylcholine, which were taken to be 100%. Values are reported as means ± SEM. The significance of the differences was calculated by the paired or unpaired Student *t*-test. Values were considered to be significantly different from control when $P<0.05$.

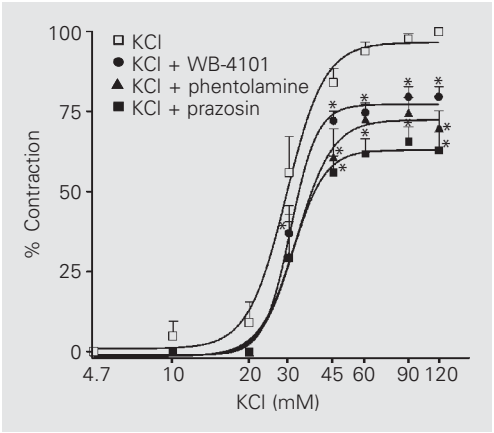

Figure 1. Effect of α-adrenoceptor antagonists on the contractile response induced by KCl. Concentration-effect curves for KCl were constructed before (control) and after incubation for 20 min with 10 nM WB-4101, 1 μM phentolamine, or 1 μM prazosin. Data are reported as means ± SEM (N = 7). *P<0.05 compared to control (paired Student *t*-test).

Drugs

Phentolamine, reserpine, noradrenaline, EGTA, prazosin, and tyramine were purchased from Sigma (St. Louis, MO, USA); acetylcholine, WB-4101, nifedipine, and L-NAME were purchased from Research Biochemicals International (Natick, MA, USA). Nifedipine was dissolved in ethanol, and all the experiments using nifedipine were carried out in an organ bath protected from light. All other drugs were dissolved in deionized water just before use. EGTA was directly dissolved in Ca^{2+}-free solution.

Results

The contractile responses induced by high concentrations of KCl were concentration dependent and stable over time. When two curves were constructed separated by a 20-min interval, the contractile responses obtained in the second curve reproduced the responses obtained in the first, demonstrating that the time used for incubation with the various antagonists did not change the contractile responses to KCl.

Effect of α-adrenoceptor antagonists on the KCl-induced contractile response

As shown in Figure 1, incubation for 20 min with 1 μM prazosin significantly reduced the maximum effect induced by KCl from 100 to 53.9 ± 10.2% (N = 7). On the other hand, in the presence of prazosin, the pD_2 values were not changed (1.6 ± 0.1 to 1.2 ± 0.2). The non-selective α-adrenoceptor antagonist phentolamine significantly reduced the maximum effect of KCl (69.7 ± 3.9%, N = 6), and also significantly reduced the sensitivity, with pD_2 values decreasing from 1.57 ± 0.01 to 1.46 ± 0.035. Similarly, the selective α_{1A}-adrenoceptor antagonist

WB-4101 (10 nM) significantly reduced the maximum effect induced by KCl (79.3 ± 3.4%, N = 6) and also reduced the pD_2 values from 1.6 ± 0.03 to 1.5 ± 0.016.

Contractile responses induced by KCl in Ca^{2+}-free solution

In the absence of extracellular Ca^{2+}, KCl induced a sustained contractile response in a concentration-dependent way. The contractile responses induced by KCl were significantly reduced in Ca^{2+}-free solution containing EGTA (30 µM) compared to the contractile responses induced in 2.5 mM Ca^{2+} Krebs (Figure 2). Both the maximum effect (50.9 ± 11.9%, N = 7) and the pD_2 values (from 1.65 ± 0.03 to 1.035 ± 0.01) were significantly decreased. As shown in Figure 2, the incubation with 1 µM prazosin in Ca^{2+}-free solution completely abolished the contractile responses induced by KCl.

Effects of nifedipine on the contractile responses induced by noradrenaline or KCl

As shown in Figure 3, the maximum effect induced by KCl was not altered by 1 µM nifedipine (97.7 ± 1.6%, N = 8). However, the sensitivity to KCl was significantly decreased by nifedipine as demonstrated by the pD_2 value which changed from 1.55 ± 0.01 to 1.43 ± 0.02. When nifedipine was combined with prazosin, the maximum effect was dramatically decreased (from 100% to 20.8 ± 11.8%, N = 7, $P<0.05$), and the pD_2 value was reduced from 1.55 ± 0.03 to 1.44 ± 0.38.

Nifedipine (1 µM) did not change the concentration-effect curves for noradrenaline (results not shown).

Effect of reserpine treatment on the contractile responses induced by KCl in isolated anococcygeus muscle

Treatment of the rats with reserpine for 3 days before the experiments significantly reduced the maximum effect induced by KCl as compared to the contractile responses induced by acetylcholine (from 339.5 ± 157.8%, N = 35, to 167.3 ± 65.5%, N = 13), and increased the pD_2 values (from 1.57 ±

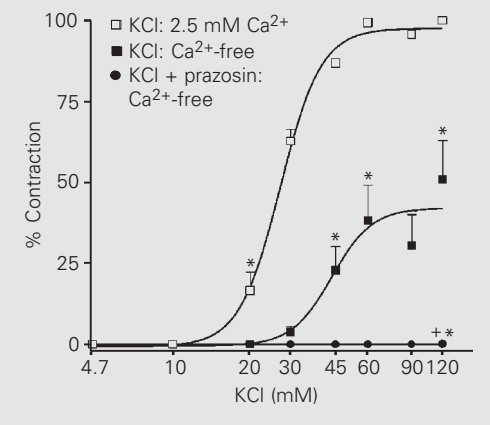

Figure 2. Effect of prazosin on the contractile responses induced by KCl in Ca^{2+}-free solution. Concentration-effect curves for KCl were constructed in 2.5 mM Ca^{2+} Krebs solution (control), and in Ca^{2+}-free solution before or after incubation with 1 µM prazosin for 20 min. Data are reported as means ± SEM (N = 7). *P<0.05 compared to control; +P<0.05 compared to responses in Ca^{2+}-free solution and in Ca^{2+}-free solution plus prazosin (paired Student *t*-test).

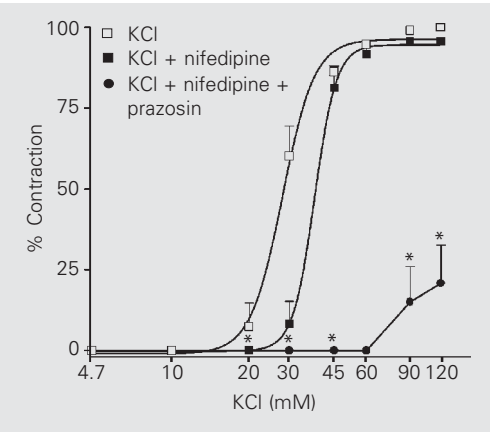

Figure 3. Effect of nifedipine, and nifedipine plus prazosin on the contractile responses induced by KCl. Concentration-effect curves for KCl were constructed before and after incubation with 1 µM nifedipine for 20 min, or with 1 µM nifedipine plus 1 µM prazosin for 20 min. Data are reported as means ± SEM (N = 8). *P<0.05 compared to responses in the absence and in the presence of the antagonists (paired Student *t*-test).

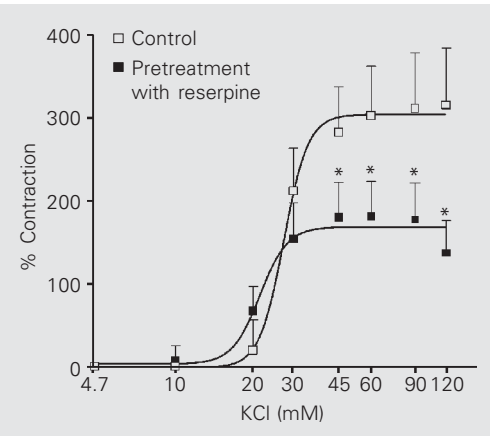

Figure 4. Effect of treatment with reserpine on the contractile responses induced by KCl. Concentration-effect curves for KCl were constructed for the anococcygeus muscle isolated from control rats (N = 35) and rats treated with 5 mg/kg reserpine (*ip*, N = 13). Data are reported as means ± SEM. *P<0.05 compared to responses by the preparations from control rats and from rats treated with reserpine (unpaired Student *t*-test).

0.01 to 1.65 ± 0.006) in preparations from treated rats, as shown in Figure 4. In preparations isolated from rats treated with reserpine in the presence of prazosin, no additive effect of prazosin was observed (Figure 5). However, incubation with L-NAME significantly increased the contractile responses induced by 120 mM KCl by 6.2 ± 2.3% (N = 7) (Figure 6). On the other hand, when the concentration-effect curves for KCl in preparations from rats treated with reserpine were constructed in Ca^{2+}-free solution, the responses were almost abolished. In fact, we observed the contractile response induced by KCl only at the concentration of 120 mM (Figure 6).

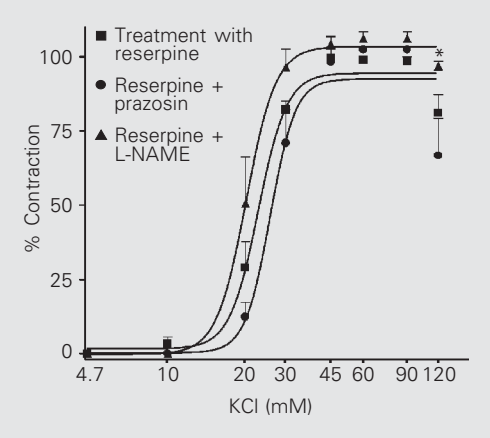

Figure 5. Effect of prazosin and L-NAME on the contractile responses induced by KCl in the anococcygeus muscle isolated from rats treated with reserpine. Concentration-effect curves for KCl were constructed for the anococcygeus muscle isolated from rats treated with 5 mg/kg reserpine (*ip*) after incubation for 20 min with 1 µM prazosin or with 100 µM L-NAME for 20 min. Data are reported as means ± SEM (N = 8). *P<0.05 compared to responses to 120 mM KCl by the preparations from control rats and from rats treated with reserpine and incubated with L-NAME (paired Student *t*-test).

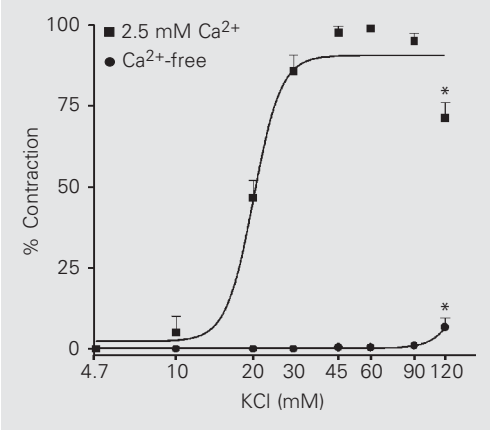

Figure 6. Effect of calcium on the contractile responses induced by KCl in the anococcygeus muscle isolated from rats treated with reserpine. Concentration-effect curves for KCl were constructed for the anococcygeus muscle isolated from rats treated with 5 mg/kg reserpine (*ip*), in 2.5 mM Ca^{2+} Krebs solution, and in Ca^{2+}-free solution. Data are reported as means ± SEM (N = 8). *P<0.05 compared to responses in 2.5 mM Ca^{2+} solution and in Ca^{2+}-free solution (paired Student *t*-test).

Discussion

The present study demonstrates that high concentrations of KCl induce the release of noradrenaline from noradrenergic neurons of the rat anococcygeus muscle.

α-Adrenoceptor antagonists inhibited the contractile response induced by KCl. The non-selective antagonist phentolamine reduced the contractile responses induced by KCl. However, its inhibitory effect was lower than the inhibitory effect of the selective α_1-adrenoceptor antagonist prazosin. As shown by Doggrell and Paton (18), in the rat anococcygeus muscle prazosin is more potent than phentolamine in antagonizing postsynaptic α_1-adrenoceptors after field stimulation or noradrenaline. We propose that KCl releases noradrenaline, which stimulates the α-adrenoceptors sensitive to prazosin in a manner similar to that induced by field stimulation. The sensitivity to KCl was not changed in the presence of 1 µM prazosin, but the efficacy was reduced.

On the other hand, the more selective α_{1A}-adrenoceptor antagonist WB-4101 reduced the maximum effect of KCl even when used at a concentration ten times lower than that of prazosin and phentolamine. Since WB-4101 produced an inhibition nearly as high as prazosin when used at a concentration that guarantees selectivity, these results suggest that α_{1A}-adrenoceptor stimulation is involved in the contractile response induced by KCl. Since WB-4101, a selective α_{1A}-adrenoceptor antagonist (22), decreased the maximal effect of KCl in a similar way as prazosin, we suggest that α_{1A}-adrenoceptor stimulation is mainly involved in the contractile response to KCl. As prazosin, a non-selective antagonist of α_1-adrenoceptors, had a slightly higher inhibitory effect, other α_1-adrenoceptor subtypes are probably also involved in the contraction elicited by KCl.

High concentrations of KCl induced concentration-dependent contractile responses which were decreased by extracellular Ca^{2+}

removal. KCl may induce the contractile responses through two mechanisms, one that is partially due to the direct depolarization of smooth muscle, and the other partially due to release of endogenous noradrenaline which interacts with postsynaptic α_1-adrenoceptors sensitive to prazosin. Our results agree with those reported by Gibson and Pollock (25), although our experimental protocol was different because we also studied these responses in Ca^{+2}-free solution.

Many intracellular biochemical reactions are highly dependent on the Ca^{2+} content in the extracellular medium. We observed that in Ca^{2+}-free solution the response induced by high concentrations of KCl was abolished by the α_1-adrenoceptor antagonist prazosin. These results indicate that in Ca^{2+}-free solution the contractile response induced by KCl is only due to the release of endogenous noradrenaline. On the other hand, in the presence of external calcium (a 2.5 mM Ca^{2+} solution), the contractile response induced by KCl was only partially inhibited by prazosin, indicating that part of the effect of KCl was probably due to a direct action on smooth muscle cells.

The Ca^{2+} channel antagonist nifedipine did not alter the contractile response induced by noradrenaline, suggesting that L-type Ca^{2+} channels sensitive to nifedipine are not involved in the contractile response induced by exogenous noradrenaline. Iravani and Zar (26) reported that nifedipine inhibits the contractions evoked by both noradrenaline and electrical field stimulation in the rat anococcygeus muscle, but the inhibition of the latter is significantly greater than the inhibition of the former. In contrast, Oriowo (27) and McGrath (28) reported that nifedipine failed to antagonize noradrenaline-induced tonic contractions of the rat anococcygeus muscle. Our results show that nifedipine reduced the sensitivity to KCl without reducing its maximum effect, but the combination of prazosin and nifedipine abolished the contractile responses induced by KCl at concentrations lower than 90 mM. It is clear that the effects of KCl in both conditions (i.e., in the presence of nifedipine plus prazosin, or in the absence of external calcium) are similar for KCl concentrations up to 60 mM. At concentrations higher than 60 mM, KCl may induce the release of other neurotransmitters like nitric oxide or acetylcholine (1,29) and 5-hydroxytryptamine (30,31) whose receptors are not sensitive to prazosin.

In the arterial mesenteric bed of the rat, 70 mM KCl releases nitric oxide (32,33). In contrast, in our studies we observed a relaxation sensitive to the nitric oxide synthase inhibitor L-NAME only in the contractile responses induced by 120 mM KCl. At lower KCl concentrations we observed contractile responses which were not sensitive to L-NAME. These results suggest that 120 mM KCl could release the NANC neurotransmitter nitric oxide in the rat anococcygeus muscle.

Reserpine causes depletion of noradrenaline stores from noradrenergic neurons. In our studies, the contractile responses induced by KCl in the anococcygeus muscle isolated from rats treated with reserpine were not inhibited by prazosin. These results demonstrate that the inhibitory effect of prazosin is only due to the antagonism of noradrenaline-stimulated receptors on the smooth muscle membrane. The contractile response induced by KCl in Ca^{2+}-free solution in the anococcygeus muscle preparations from rats pretreated with reserpine had an effect similar to that of prazosin in Ca^{2+}-free solution. These results strongly suggest that noradrenaline released from sympathetic neurons is partially involved in the responses induced by KCl.

Taken together, our results indicate that high concentrations of KCl release noradrenaline from noradrenergic neurons sensitive to reserpine, and that the released noradrenaline interacts with α_1-adrenoceptors (mainly of the α_{1A} type) in the smooth muscle cells which are sensitive to prazosin. On the other hand, KCl also produces a direct contractile response by the rat anococcygeus muscle.

References

1. Gillespie JS (1972). The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *British Journal of Pharmacology*, 45: 404-416.
2. Gibbins IL & Haller CJ (1979). Ultrastructural identification of noncholinergic, non-adrenergic nerves in the rat anococcygeus muscle. *Cell and Tissue Research*, 200: 257-271.
3. Dail WG, Carrillo Y & Walton G (1990). Innervation of the anococcygeus muscle of rat. *Cell and Tissue Research*, 259: 139-146.
4. Gillespie JS & McGrath JC (1973). The spinal origin of the motor and inhibitory innervation of the rat anococcygeus muscle. *Journal of Physiology*, 230: 659-672.
5. Burnstock G, Cocks T & Crowe R (1978). Evidence for purinergic innervation of the anococcygeus muscle. *British Journal of Pharmacology*, 64: 12-20.
6. Llinás RR & Heuser JE (1977). Depolarization-release coupling systems in neurons. *Neurosciences Research Program Bulletin*, 15: 557-687.
7. Kirpekar SM & Wakade AR (1968). Release of noradrenaline from the cat spleen by potassium. *Journal of Physiology*, 194: 595-608.
8. Blaustein MP (1975). Effects of potassium, veratridine and scorpion venom on calcium accumulation and transmitter release by nerve terminals *in vitro*. *Journal of Physiology*, 247: 617-655.
9. Turner TJ & Goldin SM (1988). Do dihydropyridine-sensitive calcium channels play a role in neurosecretion in the central nervous system? *Annals of the New York Academy of Sciences*, 522: 278-283.
10. Berridge MJ & Irvine RF (1989). Inositol phosphates and cell signalling. *Nature*, 341: 197-205.
11. Rasmussen H (1986). The calcium messenger system. *New England Journal of Medicine*, 314: 1094-1101.
12. Furuichi T, Yoshikawa S, Miyawaki KW, Maeda N & Mikoshiba K (1989). Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P_{400}. *Nature*, 342: 32-38.
13. Bülbring E & Tomita T (1987). Catecholamine action on smooth muscle. *Pharmacological Reviews*, 39: 49-96.
14. Bean BP, Sturek M, Puga A & Hermsmeyer K (1986). Calcium channels in muscle cells isolated from rat mesenteric arteries: modulation by dihydropyridine drugs. *Circulation Research*, 59: 229-235.
15. Beech DJ (1993). Inhibitory effects of histamine and bradykinin on Ca^{2+} current smooth muscle cells isolated from guinea-pig ileum. *Journal of Physiology*, 463: 565-569.
16. Gibson A & James TA (1977). The nature of potassium chloride-induced relaxations of rat anococcygeus muscle. *British Journal of Pharmacology*, 60: 141-145.
17. Bolton TB, Beech DJ & Prestwich AS (1990). Voltage and receptor gated channels. *Progress in Clinical and Biological Research*, 327: 229-243.
18. Doggrell AS & Paton DM (1978). Effects of prazosin and phentolamine on responses to field stimulation and to noradrenaline in vas deferens and anococcygeus muscle of rat. *Archives Internationales de Pharmacodynamie et de Therapie*, 233: 209-220.
19. Docherty JR & Starke K (1981). An examination of postsynaptic α-adrenoceptor subtypes in rabbit blood vessels and rat anococcygeus. *Journal of Cardiovascular Pharmacology*, 3: 854-867.
20. Coates J, Jhan U & Weetman DF (1982). The existence of a new subtype of α-adrenoceptor on the rat anococcygeus muscle is revealed by Sgd 101/75 and phenoxybenzamine. *British Journal of Pharmacology*, 75: 549-552.
21. McGrath JC (1984). Alpha-adrenoceptor antagonism by apoyohimbine and some observations on the pharmacology of alpha-adrenoceptors in the rat anococcygeus and vas deferens. *British Journal of Pharmacology*, 82: 769-781.
22. Minneman KP, Han C & Abel PW (1988). Comparison of α_1-adrenergic receptor subtypes distinguished by chlorethylclonidine and WB-4101. *Molecular Pharmacology*, 33: 509-514.
23. Gillespie JS, Liu X & Martin W (1989). The effect of L-arginine and N^G-monomethyl-L-arginine on the response of the rat anococcygeus to NANC nerve stimulation. *British Journal of Pharmacology*, 98: 1080-1082.
24. Selemidis S & Cocks TM (1997). Evidence that both nitric oxide (NO) and a non-NO hyperpolaryzing factor elicit NANC nerve-mediated relaxation in the rat isolated anococcygeus. *British Journal of Pharmacology*, 120: 662-666.
25. Gibson A & Pollock D (1973). The absence of cocaine- and 6-hydroxydopamine-induced supersensitivity to oxymetazoline in the rat anococcygeus muscle. *British Journal of Pharmacology*, 49: 726-727.
26. Iravani MM & Zar MA (1993). Differential effects of nifedipine on nerve-mediated and noradrenaline-evoked contractions of rat anococcygeus muscle. *European Journal of Pharmacology*, 250: 193-195.
27. Oriowo MA (1984). Effect of calcium-entry blockers and divalent cations on noradrenaline induced contractions of the rat anococcygeus muscle. *Archives Internationales de Pharmacodynamie et de Therapie*, 271: 45-52.
28. McGrath JC (1982). Evidence for more than one type of postjunctional α-adrenoceptor. *Biochemical Pharmacology*, 31: 467-484.
29. Gibson A & Pollock D (1975). Reduction in the cholinesterase activity of the rat anococcygeus muscle produced by corticosterone. *British Journal of Pharmacology*, 55: 69-72.
30. Goyal RK, Patel NM & Verma SC (1981). Comparative studies of the effects of 5-hydroxytryptamine and noradrenaline on the rat anococcygeus muscle. *Agents and Actions*, 11: 677-682.
31. Patel NM, Goyal RK & Verma SC (1982). Effect of uptake blockers on 5-hydroxydopamine and noradrenaline induced responses on rat anococcygeus muscle. *Indian Journal of Experimental Biology*, 20: 160-162.
32. Boric MP, Figueroa X, Donoso MV, Paredes A, Poblete L & Huidobro-Toro JP (1999). Rise in endothelium-derived nitric oxide after stimulation of rat perivascular sympathetic mesenteric nerves. *American Journal of Physiology*, 277: H1027-H1035.
33. Mendizabal VE, Poblete I, Lomniczi A, Rettori V, Huidobro-Toro JP & Adler-Graschinsky E (2000). Nitric oxide synthase-independent release of nitric oxide induced by KCl in the perfused mesenteric bed of the rat. *European Journal of Pharmacology*, 409: 85-91.