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NEUROTRANSMITTERS MEDIATING EXCITATORY RESPONSE OF BOVINE MESENTERIC ARTERY.

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ABSTRACT

The object of the present study was to shed some light on the neurotransmitters that mediate the excitatory response of the circular muscle of final branches of mesenteric artery in bovine calves. Mesentery was dissected and the iliac branches were separated and used in the experiment. The final mesenteric arteries of diameter 400 micrometers and less responded strongly to norepinephrine and moderately to ATP. However, mesenteric branches of wider diameters were gradually less responsive to norepinephrine and those of diameter 700 micrometers were exclusively nonresponsive. These arteries were strongly responsive to ATP (100 µM). Norepinephrine response was sensitive to phentolamine (3 µM) and prazosin (5 µM) indicating that it is mediated by α_1 receptor; while ATP response was sensitive to suramin (200 µM), PPADS (50 µM), but not to cibacron blue (100 µM) indicating that it is mediated via P2X receptor. Confirmative experiments were performed including application of α_1 and P2X receptor specific agonists which are methoxamine and α,β-methylene ATP. Methoxamine (1 µM) showed effects similar to norepinephrine in final branches and was without effect in wider branches. α,β-methylene ATP (1 µM), exhibited more pronounced effects on both wide and narrow branches but in parallel manner to that of ATP. Agonists for α_2 and P2Y receptors exemplified by clonidine (10 µM) and 2-meThio ATP (10 µM), respectively, were without effect indicating that involvement of these receptors is unlikely. The neuropeptide-Y (200 nM) didn't have any effects on either the narrow or the wide rings.

These data may imply that in the finest mesenteric arteries a strong pressor power represented by norepinephrine and ATP integration is needed for maintaining peripheral resistance; on the other hand a mild pressor power mediated by only ATP is needed to maintain the vascular tone in the wider mesenteric branches.

Keywords: ATP, bovine, mesenteric artery, norepinephrine.

1. INTRODUCTION

Vascular tone is regulated by various neurogenic and non-neurogenic mechanisms. The neurogenic mechanisms involve adrenergic that is well established, cholinergic that is exceptional in some species, purinergic and peptidergic that is relatively recent (5; 24; 3; 4; 16; 6). The non-neurogenic mechanisms involve circulating factors which may affect vascular dynamics, either through direct action on the vascular smooth muscle cells or through interaction with endothelial cells (12). This latter mechanism is thought to include liberation of diffusible factors from the endothelial cells which may contract vascular muscle such as endothelins A and B; or relax it such as nitric oxide and prostacycline.

Neurotransmitters controlling tone of mesenteric vessels especially purines have been studied in many species including rabbit saphenous and mesenteric arteries (7), guinea-pig mesenteric artery (18), artery of guinea-pig choroids (14), hamster mesenteric artery (25) and chicken mesenteric artery (15); in addition to norepinephrine that is well established (3). Regarding bovine mesentery, more than one study referred to the inhibitory arm of the tone (2; 1). However, there is no enough information about the excitatory arm.

Therefore, the aim of the present study was to shed some light on the excitatory mediators of mesenteric tone in bovine mesenteric artery using tension recording technique. Possible regional difference in control of bovine mesentery was also a target of the present study.

2. MATERIAL AND METHODS

2.1. Tissue preparation:

Preparations were kindly supplied by Gifu University Veterinary Hospital and were obtained from male cattle of 6 months age after their killing by exsanguinations via carotids. The mesenteric artery final branches were severed at the region of the sub-branches supplying the intestine. The vessels were transferred immediately into Kreb's solution that is aerated and its temperature was kept at 37 °C. Under a dissecting microscope, a

mesenteric branch was gently flushed with the solution and then cut into rings of 5 mm length. A ring was mounted in 10 ml-capacity organ bath filled with Kreb's solution; where 2 fine steel wires were passed through it (Figure 1). The lower wire was fixed into the bottom of the bath, while the upper one was attached to a force displacement transducer and a computer unit for recording (AD Instruments, Australia). Using a manipulator, the smooth muscle of the ring was subjected to 1 g tension. The ring was left to equilibrate for 60 min before application of drugs. For determining the receptor type that is involved in a response, the blocker was added to the bath for 10 minutes then the agonist was added and the responses before and after application of the blocker were compared.

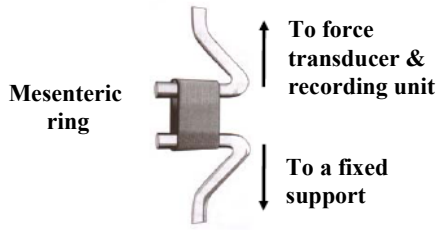


Figure 1

2. 2. Physiological solution:

The physiological solutions used in this study had the following composition in mM: NaCl 118, KCl 4.6, $CaCl_2$ 2.7, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11. The solution in the supply reservoir was gassed continuously with 95% O_2 : 5%CO_2 gas mixture creating a pH of 7.2 and was warmed to 37°C.

2.3. Drugs:

All drugs including norepinephrine (NE), adenosine 5'-triphosphate (ATP), α,β-methylene ATP (α,β-MeATP), 2-methyl-thio ATP (2-me-S-ATP), phentolamine, prazosin, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic (PPADS), cibacron blue (CB), neuropeptide Y (NPY), suramin, methoxamine and clonidine were purchased from Sigma (St. Louis, MO, USA) and were serially diluted in the PSS to the required final concentration just before the experiments.

2.4. Statistical analysis:

Data are expressed as a mean±s.e.m. of three separate arteries in which mechanical events were recorded. Statistical analysis was performed with Student's unpaired (*t*)-test, and *P* values < 0.05 were considered to be statistically significant.

3. RESULTS

3.1. General observations:

Circular muscle of bovine narrow mesenteric arteries is not quiescent muscle where it showed spontaneous activity in most of preparations especially at the start of experiments; however, when present, this activity stops after sometime. In contrast, wider branches were almost quiescent.

3.2. Responses to NE and ATP and NPY:

Arterial rings prepared from narrow mesenteric branches (Figure 2a) were responsive to NE and the contractile response was concentration-dependent (1~10 μM). On the other hand, and this is the interesting data of the present study, wider rings were almost non-responsive to NE (Figure 3a).

Application of ATP produced contractile responses in both narrow and wide arterial ring preparations (Figures 2b and 3b, respectively). Repeated application of ATP onto the arterial rings exhibited decreased responses as expected because of desensitization of purinergic receptors.

Responses similar to the actions of NE and ATP were mediated by methoxamine and α,β -MeATP, respectively (Figures 4c and 5d).

The third putative transmitter NPY was without effects in all preparations as shown in figures 2c and 3c.

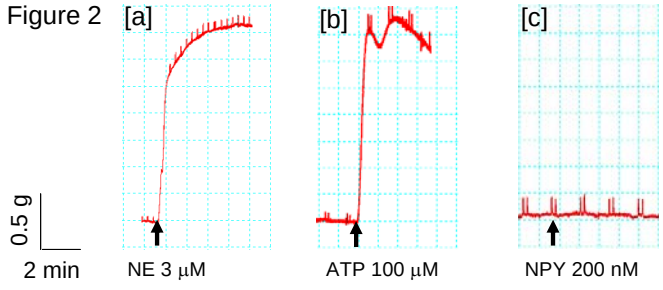
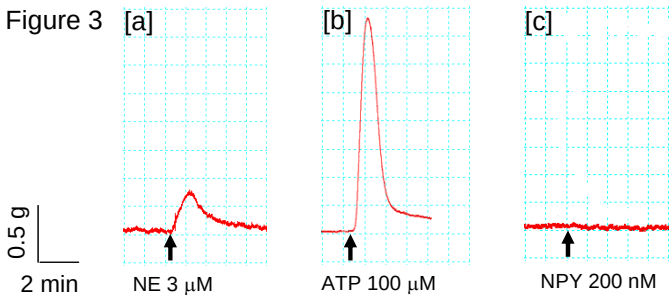
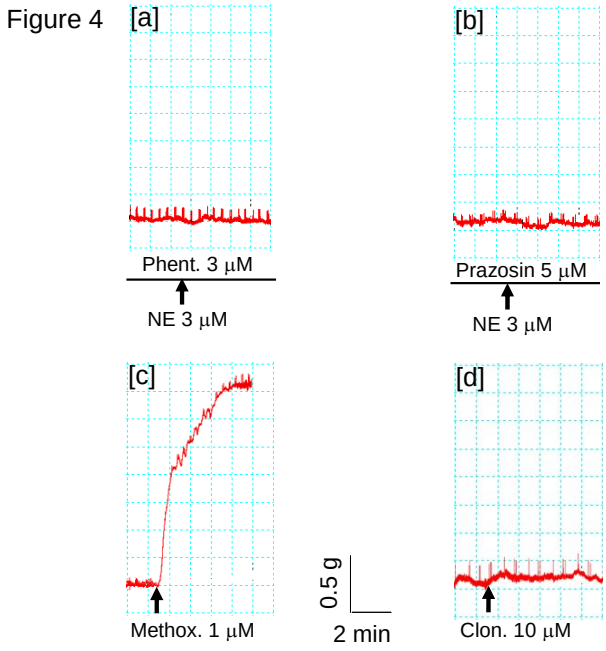


Figure 2



3.3. Adrenergic receptor identification:

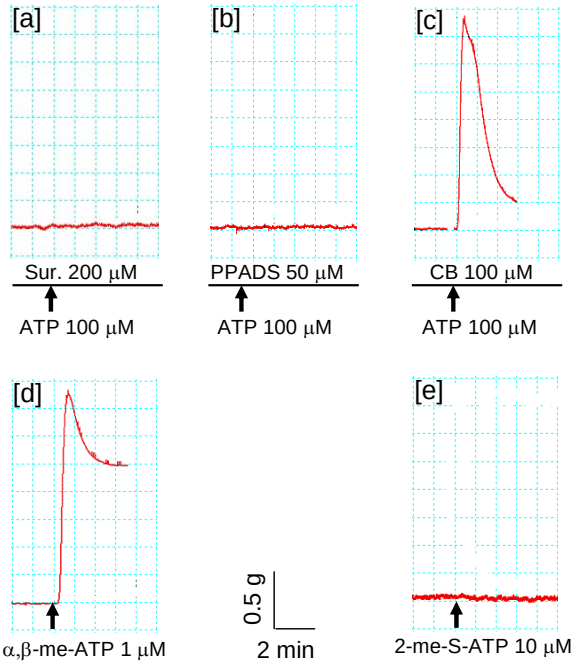
The response of the narrow arterial ring preparations to NE was blocked by the non-selective alpha adrenergic blocker phentolamine (3 μM). Prazosin, the selective α_1-adrenergic receptor blocker produced the same effect (Figures 4a and b). On the other hand, the selective α_2-agonist clonidine (10 μM) exhibited no effect (Figure 4d).



3.4. Purinerrgic receptor identification:

As shown in figure 5, the responses of the wide arterial ring preparations to ATP and α,β -Me ATP were sensitive to the P2X purinoceptor blocker PPADS (50 μM) but not to the P2Y purinoceptor blocker cibacron blue (100 μM). Similar results were obtained in narrow ring preparations (data not shown). The selective P2Y purinoceptor agonist 2-methyl-thio-ATP (10 μM) was without effect.

Figure 5



4. DISCUSSION

The present study provides evidence that NE and ATP mediate the contractile response by acting on α_1 and P2X receptors in the circular smooth muscle of bovine mesenteric artery with regional difference. Supportive lines of evidence for this conclusion are: (i) contraction of the narrow mesenteric rings but not wide ones by NE, (ii) blockade by the nonspecific α-adrenergic antagonist,

phentolamine, as well as by the specific α_1-adrenergic receptor antagonist, prazosin, which blocked the contraction evoked by NE, (iii) application of ATP and α,β-MeATP produced fast developing contraction of narrow mesenteric rings as well as wide ones, (iv) blockade by the specific P2X purinoceptor antagonist, PPADS, but not by specific P2Y purinoceptor antagonist, which blocked the contraction evoked by ATP, and (v) non-responsiveness of mesenteric rings to application of NPY.

To our knowledge, this is the first report shedding some light on the excitatory properties of the smooth muscle of bovine anterior mesenteric artery. The first characteristic property was that such smooth muscle is not quiescent as other blood vessels especially at the peripheral parts at their attachment to the ileum. This observation may indicate a stronger excitatory control in periphery than center of the mesentery.

Purinergic co-transmission involving ATP and NE has been identified and characterised through a number of lines of investigation. It had been known for many years that NE and ATP are stored together in the same sympathetic vesicles (9). However, co-existence alone is not evidence that substances have a functional role as co-transmitters. Evidence for co-transmission of NE and ATP has been provided through experiments showing that these agents are co-released from sympathetic nerves and have distinct vasomotor effects at different postjunctional receptors, which can be blocked by selective antagonists. In this respect, a compound that is widely used in studies of ATP co-transmission is α,β-meATP, a relatively stable analogue of ATP which acts as a potent and selective agonist at P2X receptors. Following activation, P2X receptors rapidly desensitize. NPY is co-localized and co-released with NE and ATP from sympathetic varicosities, but acts largely as a neuromodulator, augmenting postjunctional responses and acting prejunctionally to inhibit the release of sympathetic neurotransmitters (17).

Following ATP release from sympathetic varicosities it acts on specific P2X purine receptors, on the postjunctional membrane to evoke a change in membrane potential and a contractile response. The mechanism involves rapid membrane depolarisation (or

excitatory junction potentials, EJPs), which may summate, resulting in further membrane depolarisation, and Ca^{2+} influx via voltage-dependent Ca^{2+} channels. In electrophysiological studies, electrical stimulation of perivascular nerves elicits EJPs and slow depolarisation of the vascular smooth muscle, and both responses are abolished by guanethidine or tetrodotoxin indicating an involvement of sympathetic nerves (22; 9; 8). A role for ATP and NE in mediating the EJP and slow depolarization, respectively, is indicated by the fact that the EJP is inhibited following desensitization of P2X1 receptors by α,β-meATP, and is resistant to α-adrenoceptor blockade, while the slow depolarisation is blocked by α-adrenoceptor blockade. In addition, exogenously applied ATP mimics the EJP, while exogenously applied NE evokes a slow depolarisation. The two distinct phases of membrane depolarisation are due to the fact that actions of ATP at P2X receptors are rapid, because these receptors are ionotropic (mediate rapid Ca^{2+} influx), while actions of NE at α-adrenoceptors are slower, because these receptors are G protein-coupled and mediate their effects through second messenger systems. Pharmacological studies of vasocontractile function have shown that, as with the electrophysiological studies, distinct purinergic and noradrenergic components of vasocontraction can be identified through the use of selective antagonists. Hence, α,β-meATP blocks part, but not all, of the vasocontraction to sympathetic nerve stimulation, while the residual response can be blocked by an α-adrenoceptor antagonist, indicating an involvement of both P2X receptors and α-adrenoceptors respectively, and hence an involvement of ATP and NE.

Although the previous established physiopharmacological facts have been established in many laboratory animals, yet there is a few data regarding bovine mesentery and this was the aim of the present study. Bovine mesentery showed similarity to other species regarding involvement of ATP and NE in mesenteric vasocontractile response where exogenous application of ATP produced fast developing contractile responses in mesenteric rings prepared from iliac branches throughout their course. Our experiments also explained that ATP produces such effect via

activation of P2X receptors as its effect was blocked by prior application of the specific antagonist of P2X receptors,PPAS, but not by the specific P2Y receptor antagonist CB. The result was further confirmed by experiments using specific agonists rather than antagonists including α,β-MeATP the specific agonist of P2X receptors which produced ATP-like response. However, the specific agonist for P2Y receptor, 2-me-S-ATP, was without effect, indicating that involvement of P2Y receptors is unlikely.

Data of the present study is consistent with Burnstock and Warland (1987; 10) who used α,β-MeATP and an α-adrenoceptor antagonist (prazosin) to identify the purinergic and noradrenergic components respectively, in sympathetic perivascular nerves of the rabbit saphenous artery.

There is considerable variation in the relative proportions of ATP and NE released as functional co-transmitters in sympathetic nerves, which depends on a number of factors including species, type and size of blood vessel and experimental conditions.

Bovine mesentery was proved in this study to be a good example for this fact regarding NE, where strong vasocontractile responses was recorded only from final branches with diameter less than 400 µM. This response was gradually decreased in the wider direction until it completely disappears in rings of 700 µM diameter.

NE and ATP have been shown to be differentially released at different parameters of electrical stimulation of sympathetic nerves, with the purinergic component being favoured by relatively short trains of stimuli at lower frequencies (9; 23). Cooling has been shown to increase purinergic responses in rat mesenteric arteries (26), which might have special significance in peripheral blood vessels. The contribution of ATP to neurally evoked contractions of rat mesenteric arteries has been reported to increase as vessel diameter decreases (13). Consistent with this, in guinea-pig submucosal arterioles and in jejunal branches of the rabbit mesenteric artery, ATP has been reported to be the sole mediator of the contractile response and EJPs recorded in response to sympathetic nerve stimulation, with the role of NE being to act as a prejunctional neuromodulator via α_2-

adrenoceptors, causing depression of neurotransmitter release (20; 11). It has recently been shown that there is an important relationship between blood vessel tone/pressure and the relative proportions of ATP and NE acting as functional transmitters of sympathetic nerves. It was shown that the relative importance of ATP as a functional sympathetic neurotransmitter increased as tone/perfusion pressure was raised to physiological levels (from 30 mmHg to 90 mmHg) such that ATP became the predominant neurotransmitter (19; 21). Moreover, in the rat whole mesenteric arterial bed a purinergic component of sympathetic neurotransmission was absent at low tone and was revealed only at raised tone (19). These findings suggest that the relative importance of ATP as a co-transmitter in sympathetic nerves may have been underestimated in experiments carried out at relatively low tone/pressure. This has important implications for our understanding of purinergic transmission in vivo under normal conditions and in hypertension. Co-transmission allows an increase in the potential complexity of neurogenic responses due to differences in the time course of release, diffusion, postjunctional action, or inactivation of co-transmitters (9).

In conclusion, data of the present study provided evidence that neurotransmitters driving the mesenteric tone in bovine mesentery are mainly ATP along its whole course via P2X purinoceptor; and NE only along the peripheral mesentery via α_1-adrenoceptor.

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