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Characterization of α-Adrenoceptor Activity in Term-newborn Piglet Mesentery

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Abstract

For further characterization of neonatal mesenteric α_1-adrenoceptor populations, an extracorporeal perfusion circuit was established to control intestinal blood flow in 0–2 day old piglets.

Activation of α_1-adrenoceptors was first documented by observing dose-dependent increases in mesenteric perfusion pressure after intra-mesenteric arterial injection of methoxamine and noradrenaline. Peripheral intravenous injections of WB 4101 (a competitive α_{1A}-adrenoceptor antagonist), but not clorethylclonidine (CEC, an α_{1B}-adrenoceptor antagonist), significantly ($P < 0.05$, analysis of variance) blunted mesenteric vasoconstrictor responses to those agonists.

That the mesenteric vasoconstrictor response to mesenteric plexus stimulation was unaltered by CEC, but was muted by both WB 4101 and SK&F 104856 (a post-junctional α_1- and α_2-adrenoceptor antagonist) suggests that pre- and post-junctional α_{1A}-adrenoceptors are present and functional at birth.

Data have accumulated suggesting that the sympathetic nervous system regulates, in large measure, the adult mesenteric vascular bed (Ross 1971; Swan & Reynolds 1971) and that baseline mesenteric vascular tone can be modulated through a variety of mechanisms involving unique α-adrenoceptor populations (Patel et al 1981). Although this area of investigation has received considerable attention in adult animal models, the presence of, and extent to which, α-adrenoceptors modulate neonatal mesenteric vascular tone is largely unknown. Splanchnic nerve stimulation and blockade studies in piglets have demonstrated that the regulatory influence of the autonomic nervous system on mesenteric flow is present at birth, but increases in magnitude over the several initial weeks of life (Buckley et al 1985). Using newborn piglets others have observed mesenteric artery vasodilation in response to mild hypoxaemia, but a phentolamine-sensitive vasoconstriction when the animals were exposed to severe hypoxaemia (Gootman et al 1981). Whereas these data suggest that α-adrenoceptors play a role in the modulation of neonatal mesenteric vascular responses, they also invoke the hypothesis that mesenteric α-adrenoceptor function might undergo appreciable alterations as the animals mature.

Our laboratory has begun to identify and characterize the functional α-adrenoceptor populations in the mesenteric vascular bed of newborn (0–2 day old and 2 week old) piglets (Hoang et al 1996). We found that the mesenteric vasoconstrictor responses to the α_1-adrenoceptor agonists methoxamine and noradrenaline observed in both animal groups, were attenuated by structurally unrelated α_1-adrenoceptor antagonists (prazosin and YM 12617) in the 2-week-old piglets only. Although it appeared that mesenteric α_1-adrenoceptor-like activity in the mesentery was altered as newborn piglets matured, it was felt that a more detailed pharmacological analysis of the mesenteric α-adrenoceptor populations in 0–2 day old piglets would help clarify the mechanism(s) controlling what was observed to be a prominent vasoconstrictor response to traditional α-adrenoceptor agonist agents in that age group.

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Materials and Methods

Chemicals

All chemicals were dissolved and diluted to final concentrations in 0·9% NaCl: methoxamine (RBI, 10 mg mL^{-1}); noradrenaline (Sigma, 10 mg mL^{-1}); chlorethylclonidine (RBI, 1 mg mL^{-1}); WB 4101 (RBI, 1 mg mL^{-1}); and, SK&F 104856 (Smith-Klein, 1 mg mL^{-1}). To ensure freshness all compounds were frequently reconstituted from their respective stocks and kept refrigerated (4°C) when not in use.

Surgical preparation and instrumentation

All experimental procedures were completed in accordance with the guidelines and approval of the Tulane University School of Medicine Advisory Committee for Animal Resources.

Standard breed 0–2-day old piglets of either sex (average age 1·3 ± 0·5 days; average weight = 1·3 ± 0·05 kg) were brought to the laboratory on the day of the experiment. The animals were placed in a warmed environment and observed in a fasted state for 4 h. Piglets with obvious physical illnesses, including diarrhoea and lethargy, were excluded. Each piglet was anaesthetized with intraperitoneal pentobarbital sodium (35 mg mL^{-1}). Intraperitoneal pancuronium bromide (0·5 mg mL^{-1}) was administered in order to perform tracheotomy and intubation. The animal was placed on a ventilator (Harvard Apparatus) preset to deliver: 8–12 mL kg^{-1} tidal volume; 20–30 breaths min^{-1}; 1·0 inspired oxygen content; 5 cm H_2O positive end expiratory pressure. These settings were adjusted to maintain serially obtained arterial blood gas values within the ranges: pH = 7·35–7·45, PCO_2 = 35–45 mmHg, PO_2 > 500 mmHg. External heating sources were used to maintain a rectal temperature of 38 ± 0.5°C.

Through a groin incision, the left femoral vein was cannulated to administer maintenance intravenous fluid (1–2 mL $kg^{-1} h^{-1}$ lactated Ringer's solution) and to provide access for a pentobarbital sodium infusion that was adjusted (15–30 $mg\,kg^{-1} h^{-1}$) to maintain the animal in a surgical plane of anaesthesia. The left femoral artery was cannulated and connected to a pressure transducer (Statham model P23) for continuous recording of mean arterial pressure (Grass model 7D polygraph) and for periodic blood gas sampling. The right femoral artery was exposed through a groin incision.

The cranial mesenteric artery (equivalent to the superior mesenteric artery) was identified via a left flank retroperitoneal approach and was gently dissected from surrounding connective tissue. An extracorporeal circuit was prepared by first priming the infusion tubing of a perfusion pump (Harvard Apparatus model 1210) with heparinized saline. Heparin sodium (1000 $U\,kg^{-1}$) was intravenously administered to the animal, after which a right femoral arteriotomy was made and the tip of a cannula was positioned in the distal abdominal aorta. The opposite end of the cannula was connected to the inlet side of the pump circuitry. The cranial mesenteric artery was ligated at its point of origin from the abdominal aorta, and a cannula was inserted through an arteriotomy fashioned just distal to the ligature. This cannula was connected to the outlet of the perfusion circuitry, and flow to the intestine rapidly re-established at a constant rate by the perfusion pump. A lateral tap, positioned between the pump and the cranial mesenteric artery, was connected to a pressure transducer to enable continuous recording of the mesenteric perfusion pressure.

By manipulating the perfusion pump rate, the baseline mesenteric flow (mean flow $= 35{\cdot}9 \pm 1{\cdot}8$ mL min^{-1}) was adjusted so that the mean mesenteric perfusion pressure approximated the mean systemic arterial pressure (mean pressure $= 44 \pm 0{\cdot}9$ mmHg), and kept constant throughout the experiment. Thus, an increase in mesenteric perfusion pressure directly reflected an increase in mesenteric vascular resistance. Before instituting each experiment, a 10–20 min time-interval was typically needed to ensure that mesenteric perfusion pressure, systemic mean arterial pressure, heart rate, and arterial blood gas variables were unchanged for two consecutive measurements taken 5 min apart.

In all animals, α_1-adrenoceptor agonists were first bolus injected into the cranial mesenteric artery-in small volumes (10–30 μL), in random sequence, and in varying concentrations-to define the vasoconstrictor responses in a dose-dependent fashion. Each subsequent injection was made after leaving sufficient time (5–10 min) for the observed mesenteric vasoconstrictor response to resolve, with a return of the mesenteric perfusion pressure to pre-administration baseline levels. To determine the selectivity of the α-adrenoceptor-mediated mesenteric vasoconstrictor response, α-adrenoceptor antagonists were then intravenously infused over 20 min (to minimize alterations in mean systemic arterial pressure) before intra-mesenteric arterial bolus injections of the previously administered agonist agents.

Experimental protocols

Zero to two day old piglets ($n = 6$ $group^{-1}$) were submitted to one of the protocols: (Group 1) intra-mesenteric arterial injection of methoxamine and noradrenaline before and after intravenous injection of WB 4101 (a selective α_{1A}-adrenoceptor antagonist (Minneman et al 1988); 0·5 $mg\,kg^{-1}$), and again after chlorethylclonidine (CEC, an irreversible α_{1B}-adrenoceptor antagonist (Minneman et al 1988); 1 $mg\,kg^{-1}$); (Group 2) repetition of group 1 protocol, reversing the order of WB 4101 and CEC.

To define the extent to which endogenous mesenteric vasoconstrictor activity was functional in 0–2 day old piglets, other animals were subjected to mesenteric nerve plexus stimulation experiments. The cranial mesenteric nerve plexus was isolated and proximally ligated after mesenteric perfusion circuitry had been established. Fibres proximal to the ligature were crushed to ensure decentralization. The distal plexus fibres were placed on a shielded Harvard electrode and stimulated (Grass nerve stimulator model SD9) by applying a 10 s train of pulses (10 V amplitude, 5 ms duration). Baseline frequency–response data were generated by determining the mean increase in mesenteric perfusion pressure at 10 Hz and 30 Hz, after which the following protocols were conducted: (Group 3) mesenteric nerve stimulation before and after intravenous injection of WB 4101, then again after peripheral intravenous injection of CEC, then again after peripheral intravenous injection of SK&F 104856 (a postjunctional α_1- and α_2-adrenoceptor antagonist, 1 $mg\,kg^{-1}$); (Group 4) repetition of group 3, altering the order of antagonists to SK&F 104856, CEC, then WB 4101; (Group 5) repetition of group 3, altering the order of antagonists to CEC, WB 4101, then SK&F 104856.

Data analysis

For each perturbation, the peak increases in mean mesenteric perfusion pressure were converted to percent increases from baseline mean mesenteric perfusion pressure. Within- and between-group mean values ($\pm 1 \times$ s.e.) were subjected to analysis of variance. When a significant F ratio was reached a Newman-Keuls test was applied to detect specific differences. Significance was accepted when $P < 0{\cdot}05$.

Results

The effects of peripheral intravenous infusions of WB 4101 and CEC on the mesenteric vasoconstrictor responses observed in piglets given methoxamine, an α_1-adrenoceptor agonist, by intra-mesenteric arterial bolus injection are shown in Table 1. Intra-mesenteric arterial administration of methoxamine produced a dose-dependent increase in mesenteric perfusion pressure. WB 4101, a selective α_{1A}-adrenoceptor antagonist, dramatically blocked this mesenteric vasoconstrictor response. The subsequent administration of CEC, an irreversible α_{1B}-adrenoceptor antagonist, failed, however, to produce a significant additional blocking effect. When the order of WB 4101 and CEC infusions was reversed in separate groups of piglets, CEC still failed to produce a significant blocking effect on the mesenteric vasoconstrictor response to methoxamine. That the subsequent infusion WB 4101 did block this effect strongly suggests that α_{1A}-adrenoceptors, not α_{1B}-adrenoceptors, specifically mediate the mesenteric vasoconstrictor response in this age group.

The effects of WB 4101 and CEC on noradrenaline, a mixed α_1- and α_2-adrenoceptor agonist in which the α_1-type predominates, on the mesenteric vasoconstrictor response are shown in Table 1. Once again, WB 4101 significantly blocked

Table 1. Effects of peripheral intravenous WB 4101 on the vasoconstrictor response to intramesenteric arterial methoxamine or noradrenaline, when given before, or subsequent to, peripheral intravenous CEC.

	Methoxamine		Noradrenaline	
	10 mg kg^{-1}	30 mg kg^{-1}	10 ng kg^{-1}	30 ng kg^{-1}
Control	10·97 ± 0·40*	25·90 ± 0·63	9·91 ± 1·10*	17·54 ± 1·30
WB 4101	0·97 ± 0·47†	2·70 ± 0·78†	3·71 ± 0·90†	8·27 ± 1·50†
WB 4101/CEC	0·63 ± 0·48†	3·22 ± 1·47†	2·55 ± 1·40†	6·17 ± 1·50†
Control	11·40 ± 1·20*	26·50 ± 1·60	10·70 ± 0·48*	17·52 ± 0·57
CEC	9·74 ± 1·08	23·67 ± 2·13	8·88 ± 1·03	15·34 ± 1·20
CEC/WB 4101	1·35 ± 0·85†‡	3·57 ± 1·39†‡	4·02 ± 0·99†‡	6·45 ± 1·95†‡

*$P < 0.001$ compared with 30-μg or 30 mg dose. †$P < 0.05$ compared with control. ‡$P < 0.05$ compared with CEC.

the dose-dependent mesenteric vasoconstrictor response to noradrenaline; the subsequent administration of CEC produced no further blocking effect. When the order of antagonist agents was reversed, only WB 4101 produced a significant blunting of the mesenteric vasoconstrictor response to noradrenaline, confirming the functional presence of α_{1A}-adrenoceptor, not α_{1B}-adrenoceptor, subpopulations in term-newborn piglets.

Having shown that a mesenteric vasoconstrictor response could be generated by exogenous stimulation, we wished to determine whether, and the extent to which, endogenous mechanisms (e.g. the autonomic nervous system) that are known to initiate this response in adult animal models were operative in newborn piglets. As shown in Table 2, electrical stimulation of the cranial mesenteric nerve fibres produced a frequency-dependent increase in mesenteric perfusion pressure, suggesting that newborn piglets possess an intact and functional mesenteric autonomic nervous system. The effects of peripheral intravenous injections of WB 4101, CEC, then SK&F 104856 (primarily a post-junctional α_1- and α_2-adrenoceptor antagonist) on the mesenteric vasoconstrictor response to nerve stimulation in newborn piglets were then examined. Once again, WB 4101 blunted the mesenteric vasoconstrictor response. The subsequent administration of CEC caused no additional blocking effect, indicating the presence of α_{1A}- but not α_{1B}-adrenoceptors. That SK&F 104856 produced even further inhibition of this vasoconstrictor response signifies the presence of functional post-junctional α-adrenoceptors in newborn piglets.

Table 2. Effects of peripheral intravenous WB 4101, CEC and SK&F 104856, given in various sequences, on the mesenteric vasoconstrictor response to nerve stimulation.

	Mesenteric plexus stimulation	
	10 (Hz)	30 (Hz)
CONTROL	10·50 ± 1·52*	23·00 ± 1·00
WB 4101	6·17 ± 2·79†	15·67 ± 1·86†
WB 4101/CEC	5·10 ± 2·14†	13·00 ± 1·41†
WB 4101/CEC/SKF	2·12 ± 1·76†‡	7·00 ± 2·45†‡
CONTROL	21·44 ± 0·82*	44·30 ± 1·25
CEC	19·16 ± 2·80	40·00 ± 0·95
CEC/WB 4101	7·36 ± 1·11†§	16·80 ± 1·20†§
CEC/WB 4101/SKF	2·04 ± 0·74†‡	4·60 ± 1·40†‡
CONTROL	21·45 ± 0·99*	40·99 ± 0·74
SKF	9·36 ± 2·51†	22·57 ± 3·94†
SKF/CEC	7·33 ± 1·49†	17·70 ± 2·00†
SKF/CEC/WB 4101	3·69 ± 1·89†**	12·00 ± 3·43†**

*$P < 0.0001$ compared with 30 Hz frequency. †$P < 0.05$ compared with control. ‡$P < 0.05$ compared with WB 4101, CEC. §$P < 0.05$ compared with CEC. **$P < 0.05$ compared with SK&F 104856, CEC.

When the order of blocker agents was altered, giving CEC, WB 4101, then SK&F 104856 by peripheral intravenous infusion, CEC produced no effect on the mesenteric vasoconstrictor response, indicating a lack of appreciable α_{1B}-adrenoceptor activity. In contradistinction, WB 4101, then SK&F 104856, each caused significant decreases in the mesenteric vasoconstrictor response to nerve stimulation, providing evidence of functional pre-junctional α_{1A}-adrenoceptors in newborn piglet mesentery. In the final experimental protocol, the order of antagonist agent infusions was changed to SK&F 104856, CEC, and WB 4101. Postjunctional α_1-adrenoceptor activity in newborn piglet mesentery was clearly demonstrated by the significant blocking effect of SK&F 104856. That CEC once again had no obvious effect on the mesenteric vasoconstrictor response to nerve stimulation reaffirms the dearth of functional α_{1B}-adrenoceptors. The subsequent administration of WB 4101 induced even further inhibition of the mesenteric vasoconstrictor response in these animals, signifying that prejunctional α_{1A}-adrenoceptors are present in the mesentery of newborn piglets.

Discussion

Data reported herein reaffirm our previous finding that, at birth, term piglets possess the ability to generate a mesenteric vasoconstrictor response to classic, exogenously administered, α_1-adrenoceptor agonist agents. Because it is now well established by a variety of recently completed in-vitro molecular cloning studies that there are at least three, perhaps four, subtypes of α_1-adrenoceptor (Bylund 1988), we sought to define the extent to which some of these adrenoceptor subpopulations were functional in this age group of animals. That intravenous administration of WB 4101, but not CEC, blocked the mesenteric vasoconstrictor response to two structurally unrelated α_1-adrenoceptor compounds (methoxamine and noradrenaline) clearly indicates that α_{1A}-adrenoceptors, and not α_{1B}-adrenoceptors, are present at birth.

These data might appear at odds with our previous report that prazosin, a known α_1-adrenoceptor antagonist, did not blunt the mesenteric vasoconstrictor response to methoxamine

or noradrenaline in 0–2-day old piglets (Hoang et al 1996). In general, prazosin has been found to possess similar affinities for the known α_1-adrenoceptor subpopulations. Others have, however, found that in certain tissues prazosin does demonstrate a selective α-adrenoceptor subpopulation profile. For example, Mignot et al (1989) used radioligand binding techniques to show that in dog brain prazosin bound to two different α-adrenoceptor sites, although there was a thirtyfold difference in affinity between the two. On the other hand, these authors found only one prazosin-sensitive binding site in rat and monkey brain. Using the same experimental technique, Klijn et al (1991) have shown that, whereas rat renal cortical cells have equal amounts of α_{1A}-and α_{1B}-adrenoceptors, only prazosin-sensitive α_{1B}-adrenoceptor binding sites were identified in a distal tubular cell line. Finally, Oriowo et al (1989) have studied in-vitro contractile responses of rat and cat arteries to noradrenaline, and have shown that the sensitivity to this agonist was quite variable. The authors concluded that these differences in sensitivity can be best attributed to a change in the affinity for each of the α-adrenoceptor subpopulations. An alternative explanation of their observations was offered by Bylund (1988), who suggested that blood vessels might simply have different concentrations of α_1-adrenoceptor subtypes. When data from our experiments are analysed within this context, it appears likely that the 0–2-day old piglet mesenteric arterial system has functional α_{1A}-adrenoceptors that have low affinity for prazosin binding.

The present experiments also demonstrate that an α_{1A}-adrenoceptor subpopulation is not only present in the mesentery of term-newborn piglets at birth, but is also subject to endogenous regulatory control through the autonomic nervous system. These data are in agreement with reports that newborn piglet mesenteric blood flow is under autonomic control at birth (Buckley et al 1985). The present data demonstrate, however, that the autonomic nervous system regulates term-newborn piglet mesenteric blood flow through a mature, selective process that is mediated at least through α_{1A}- and not α_{1B}-adrenoceptors.

The reduction in the mesenteric vasoconstrictor response to autonomic nervous system stimulation by SK&F 104578, a selective postjunctional α_1-adrenoceptor antagonist (Hieble et al 1992), indicates the presence of postjunctional α_1-adrenoceptors at birth in piglets. When, moreover, WB 4101 and SK&F 104856 were administered to the same piglet, each resulted in further blunting of the mesenteric vasoconstrictor response to autonomic stimulation, irrespective of the order in which these agents were given. These data clearly indicate that term-newborn piglets do possess a sophisticated system of prejunctional and postjunctional α_{1A}-adrenoceptors that are under regulatory control of the autonomic nervous system in piglet mesentery.

In all, our data strongly suggest that at birth term-newborn piglets have an elaborate, fairly selective α_1-adrenoceptor system that controls mesenteric blood flow. These observations have particular clinical import as one attempts to define strategies to induce selective mesenteric vasodilation when an infant is placed under circumstances in which prolonged mesenteric vasoconstriction threatens intestinal viability.

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