

Research Article

Influence of Hydroxocobalamin and Carboxy-PTIO on Nitric Oxide-Mediated Signaling in Porcine Anococcygeus and Retractor Penis Muscles

Hari Prasad Sonwani*

Apollo College of Pharmacy, Durg, Chhattisgarh-491001, India

Corresponding author: Hari Prasad Sonwani, Apollo College of Pharmacy, Durg, Chhattisgarh-491001, India
Email: harisonwani10@gmail.com**Received:** May 02, 2025**Accepted:** June 09, 2025**Published:** June 11, 2025**Abstract**

this study examined the influence of carboxy-PTIO and hydroxocobalamin on nitrgic neurotransmission in porcine anococcygeus and retractor penis muscles obtained post mortem from young male pigs. Under basal conditions, electrical field stimulation (EFS) induced tetrodotoxin-sensitive contractions in both muscle types, which were significantly reduced by prazosin and guanethidine, but unaffected by atropine. In the anococcygeus muscle, guanethidine also elicited a sustained contractile response.

Following pre-contraction with guanethidine (anococcygeus) or phenylephrine in the presence of guanethidine (retractor penis), EFS evoked relaxations that were abolished by L-NAME and partially restored by L-arginine, indicating mediation by nitrgic nerves. Carboxy-PTIO (0.1–1 mM) had no significant effect on these nerve-evoked relaxations. In contrast, hydroxocobalamin (0.1–1 mM) produced a concentration-dependent inhibition of nitrgic relaxations in both muscles. Relaxation responses to exogenous nitric oxide were abolished by both carboxy-PTIO and hydroxocobalamin.

No significant differences were observed in drug responses between the two muscle types. These results support previous findings that the endogenous nitrgic transmitter exhibits resistance to NO scavenging by carboxy-PTIO, while being sensitive to hydroxocobalamin.

Keywords: Anococcygeus; L-arginine; Hydroxocobalamin; Guanethidine; Tetrodotoxin

Background and Rationale

Much of the foundational research establishing nitrgic transmission at autonomic neuroeffector junctions was conducted using the rat anococcygeus and bovine retractor penis muscles. These tissues were among the first in which peripheral nitrgic signaling was identified [1-4].

Although both muscles clearly demonstrate nitrgic neurotransmission, notable differences in their responses to pharmacological agents have been reported. Early studies showed that the nitric oxide synthase (NOS) inhibitor NG-monomethyl-L-arginine (L-NMMA) effectively suppressed nitrgic responses in the rat anococcygeus [1,5], but not in the bovine retractor penis [1]. In fact, L-NMMA—similar to L-arginine—could even restore nitrgic relaxation in bovine tissue previously blocked by NG-nitro-L-arginine [2].

More recent findings indicated that hydroxocobalamin, an NO-binding agent, significantly reduced nitrgic relaxations in the bovine retractor penis muscle [6], but not in the rat anococcygeus at concentrations sufficient to block responses to exogenous NO [5,7]. Similarly, carboxy-PTIO—a nitric oxide scavenger—failed to inhibit nitrgic responses in rodent anococcygeus muscles [8,9], while it effectively blocked them in bovine retractor penis tissue [6].

These contrasting outcomes raise the question of whether the observed differences are attributable to species-specific factors or intrinsic differences between the two muscle types. However, a true anatomical distinction is unlikely, as the retractor penis muscle is anatomically continuous with the anococcygeus muscle [2]. In fact, the anococcygeus muscles converge to form the retractor penis muscle, which extends anteriorly to the penis shaft. This muscle

structure is absent in rabbits and primates and is underdeveloped in rats. Both muscles receive sympathetic noradrenergic excitatory and parasympathetic non-adrenergic, non-cholinergic (NANC) inhibitory innervation (Sjöstrand & Klinge, 1995). Despite their structural continuity, anatomical similarities alone cannot account for the functional differences in neurotransmission.

In this study, we utilized freshly isolated anococcygeus and retractor penis muscles from post mortem pigs, available through an unrelated experimental series.

Methods

Anococcygeus and retractor penis muscles were harvested post mortem from young male pigs (40–50 kg) that had served as control animals in a separate vaccine development study. Animals were humanely stunned and exsanguinated, and tissue samples were collected during necropsy.

Muscles were immediately placed in cold, oxygenated physiological salt solution (PSS) and transported to the laboratory. Longitudinal muscle strips (2–3 mm wide, ~1 cm long) were mounted in 10 mL organ baths under 1 g of resting tension, following protocols established for the rat anococcygeus muscle [1,5]. All procedures were approved by the Royal Melbourne Institute of Technology Animal Ethics Committee.

Isometric tension changes were recorded in response to intrinsic nerve stimulation via electrical field stimulation (EFS), as well as to exogenously applied nitric oxide (NO). The impact of NO-modulating agents was evaluated after basal tone was elevated (see Results).

EFS-induced nitrgic relaxations were elicited using 1 ms pulses at supramaximal voltage, delivered at 2 Hz for 10 seconds every 2 minutes via a pair of platinum electrodes flanking the tissue. Time-matched control experiments were performed on tissue from the same animals without any nitrgic-interacting drugs.

The PSS composition was (in mM): NaCl 118, KCl 4.7, NaHCO₃ 25, MgSO₄ 0.45, KH₂PO₄ 1.03, CaCl₂ 2.5, D-glucose 11.1, and disodium EDTA 0.067.

The following pharmacological agents were used: L-arginine, atropine sulfate, guanethidine sulfate, hydroxocobalamin hydrochloride, NG-nitro-L-arginine methyl ester (L-NAME), L-phenylephrine hydrochloride, and tetrodotoxin (TTX) (Sigma, USA); carboxy-PTIO (Sapphire Bioscience, Australia); and prazosin hydrochloride (Pfizer, USA). A 2 mM NO stock solution was prepared from compressed nitric oxide gas (Commonwealth Industrial Gases, Melbourne, Australia) according to established methods [7].

All data are presented as mean \pm standard error. Statistical significance between groups was assessed using Student's *t*-test or one-way analysis of variance (ANOVA). A *P* value less than 0.05 was considered statistically significant.

Results

Figure 1 and Figure 2.

Discussion

The study by Li and Rand [5] investigated the effects of

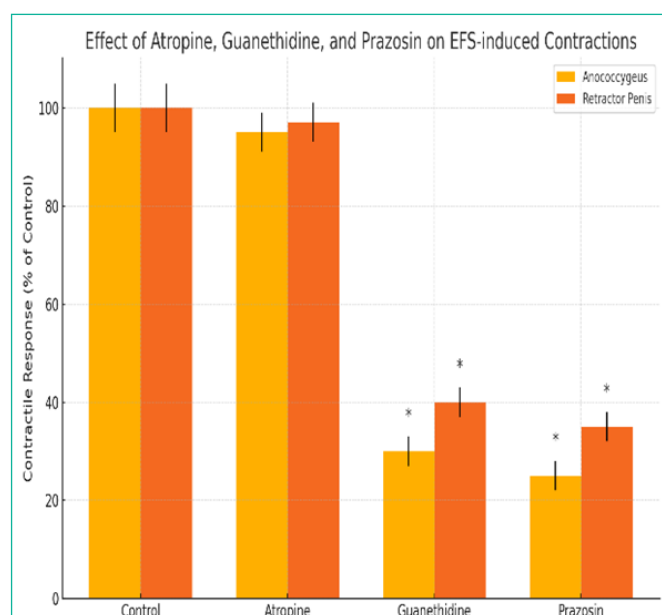


Figure 1: (A) Tracings illustrating the effects of atropine and guanethidine on field stimulation-induced contractile responses of the pig anococcygeus and retractor penis muscle. (B) Mean data for the effects of atropine, guanethidine and prazosin on field stimulation-induced contractions of the pig anococcygeus and retractor penis muscles. Columns represent means and I-bars indicate the s.e.mean of 4–5 experiments. *Indicates a significant difference ($P < 0.05$, Student's *t*-test) from the corresponding control group.

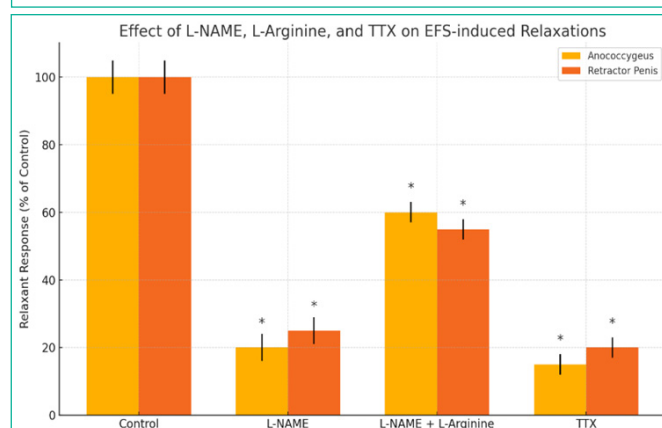


Figure 2: Here's the graph showing how L-NAME, L-NAME plus L-arginine, and tetrodotoxin affect electrical field stimulation-induced relaxations in the pig anococcygeus and retractor penis muscles. Asterisks mark statistically significant differences from control ($P < 0.05$). Would you like this exported as an image or formatted for a figure legend.

hydroxocobalamin and carboxy-PTIO on nitrgic transmission in porcine anococcygeus and retractor penis muscles. Their findings revealed that hydroxocobalamin caused concentration-dependent reductions in nitrgic relaxations in both muscles, whereas carboxy-PTIO did not significantly affect stimulation-induced nitrgic relaxations.

These results suggest that the nitrgic transmitter in these muscles is generally resistant to the NO-scavenger carboxy-PTIO, indicating a potential species-specific difference in the sensitivity of nitrgic transmission to NO-scavengers.

The study also noted that the contractile responses to electrical field stimulation (EFS) in both muscles were largely noradrenergic

in origin, as they were greatly inhibited by the $\alpha 1$ -adrenoceptor antagonist prazosin and by tetrodotoxin. Additionally, EFS-induced relaxations were abolished by the NO synthase inhibitor L-NAME, and this effect was partly reversed by L-arginine, indicating that these relaxations are mediated by nitrergic nerves.

Overall, the findings highlight the complexity of nitrergic transmission and its modulation by different agents, emphasizing the need for further research to fully understand the mechanisms involved [10-15].

Conclusion

In conclusion, the results reveal that there are no significant differences in reactivity to carboxy-PTIO or hydroxocobalamin between anococcygeus and retractor penis muscles derived from the same species (pig), supporting the notion that previously observed variations between rat and bovine tissues are primarily due to species-specific factors rather than intrinsic muscle-type differences. These findings reinforce the hypothesis that the nitrergic transmitter involved in these smooth muscle relaxations is generally resistant to scavenging by carboxy-PTIO. This resistance suggests either a protective mechanism conferred by endogenous antioxidant substances, such as ascorbate or superoxide dismutase, or that the actual transmitter may not be the free radical form of nitric oxide (NO•) traditionally assumed.

Furthermore, the concentration-dependent inhibitory effects of hydroxocobalamin on nitrergic nerve stimulation in both pig muscles confirm its role as an effective NO-sequestering agent, while also highlighting tissue-specific sensitivities to such agents. The data support the growing view that nitrergic neurotransmission may involve complex interactions between NO, its derivatives, and local tissue environments. These findings underscore the importance of considering species-specific biochemical and neurochemical environments in pharmacological studies and may have implications for the therapeutic targeting of nitrergic pathways in different species, including humans.

Further research is warranted to clarify the precise identity and regulation of the nitrergic transmitter and to determine the molecular basis for its protection from scavengers like carboxy-PTIO.

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