The following is the abstract of the article discussed in the subsequent letter:

Naik, Jay S., Zoran Valic, John B. Buckwalter, and Philip S. Clifford. Rapid vasodilation in response to a brief tetanic muscle contraction. J. Appl. Physiol. 87(5): 1741–1746, 1999.—To test the hypothesis that vasodilation occurs because of the release of a vasoactive substance after a brief muscle contraction and to determine whether acetylcholine spillover from the motor nerve is involved in contraction-induced hyperemia, tetanic muscle contractions were produced by sciatic nerve stimulation in anesthetized dogs (n = 16), instrumented with flow probes on both external iliac arteries. A 1-s stimulation of the sciatic nerve at 1.5, 3, and 10 times motor threshold increased blood flow above baseline (P < 0.01) for 20, 25, and 30 s, respectively. Blood flow was significantly greater 1 s after the contraction ended for 3 and 10 X motor threshold (P < 0.01) and did not peak until 6–7 s after the contraction. The elevations in blood flow to a 1-s stimulation of the sciatic nerve and a 30-s train of stimulations were abolished by neuromuscular blockade (vecuronium b). The delayed peak blood flow response and the prolonged hyperemia suggest that a vasoactive substance is rapidly released from the contracting skeletal muscle and can affect blood flow with removal of the mechanical constraint imposed by the contraction. In addition, acetylcholine spillover from the motor nerve is not responsible for the increase in blood flow in response to muscle contraction.

Effect of Vecuronium on the Release of Acetylcholine After Nerve Stimulation

To the Editor: The recent article by Naik et al. (8) suggests that during a brief tetanic contraction of skeletal muscle, a vasoactive substance is released that results in vasodilation and thus contributes to the rapid increase in blood flow after the muscle contraction. The authors concluded that the acetylcholine released from the motor nerve ending in response to nerve stimulation is not this vasoactive substance, as muscle blood flow did not change in response to nerve stimulation during neuromuscular blockade. The neuromuscular blockade was produced by vecuronium bromide, which was assumed to have no effect on the release of acetylcholine in response to nerve stimulation.

However, in our opinion, this assumption may not be correct. In general, it is believed that a nondepolarizing muscle relaxant such as vecuronium bromide binds not only to the postjunctional sites at the muscle membrane but also to the presynaptic sites at the nerve ending (1, 2, 4, 6, 9, 10). By binding to presynaptic acetylcholine receptors, the muscle relaxant may diminish the release of acetylcholine from the motor nerve during tetanic stimulation (2).

Therefore, the possible contribution of acetylcholine release to the increase in blood flow cannot be excluded on the basis of the described experiment. In general, the diminution in acetylcholine release is determined by the degree of presynaptic block, which may vary for each study performed. Thus the presynaptic block in the experiments performed by Naik et al. (8) may have been greater than that in the experiments performed by Welsh and Segal (11). Welsh and Segal showed vasodilation in the retractor muscle of anesthetized hamsters after nerve stimulation in the presence of tubocurarine chloride, another nondepolarizing muscle relaxant. The observed vasodilation was possibly the result of the action of the acetylcholine released from the nerve ending. Furthermore, the presynaptic block in the experiments performed by Dyke et al. (5) may have been great enough to prevent the possible action of acetylcholine on vessel diameter and blood flow. In these experiments, pipecuronium bromide was used, which is also a nondepolarizing muscle relaxant. Although pipecuronium bromide has a low affinity for presynaptic acetylcholine receptors, it may produce a presynaptic block, especially when muscles are profoundly paralyzed, as in the experiments described (3, 12).

To determine whether the release of acetylcholine from the motor nerve is involved in the increase in blood flow that follows muscle contraction, we suggest a study design using α-bungarotoxin instead of a nondepolarizing relaxant such as vecuronium. α-Bungarotoxin prevents muscle contraction without affecting acetylcholine release from the nerve ending by binding selectively to the postjunctional acetylcholine receptors (7).

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with postjunctional receptors, and binding to prejunc-
tional receptors could reduce the amount of acetylcho-
line released at the motor nerve terminals. We ac-
knowledge that our conclusion from that “acetylcho-
line spillover from the motor nerve is not responsible for
the increase in blood flow in response to muscle con-
traction” requires the neuromuscular blocker em-
ployed to have negligible prejunctional effects. Al-
though we considered making the argument that
vecuronium has a low affinity for prejunctional recep-
tors, van Santen and Wierda kindly provided the opti-
mal solution to the problem by suggesting further
studies using α-bungarotoxin, which irreversibly binds
postjunctional receptors with no detectable prejunc-
tional effects (2). Because of the importance of this
finding to an understanding of exercise hyperemia, we
performed the proposed studies using the methods
described in the original paper.

Tetanic muscle contractions were produced by sciatic
nerve stimulation in three anesthetized dogs. The
hindlimb blood flow responses to a 1-s stimulation of
the cut sciatic nerve (30 Hz, 0.1 ms, 10 × motor thresh-
old) and a 30-s train of contractions (50% duty cycle)
were determined in duplicate. As expected, the train of
contractions used to mimic dynamic exercise produced
a larger increment in hindlimb blood flow than a 1-sec
tetanic contraction (Fig 1, left). Because of the gradual
development of neuromuscular blockade after intrave-
rous administration of α-bungarotoxin (0.2 mg/kg), a
1-h interval was allowed before the nerve stimulations
were repeated. At that time, mean arterial pressure
had increased by an average of 12 mmHg, and baseline
blood flow was slightly reduced (Fig. 1). Sciatic nerve
stimulation still produced barely visible contractions in
one dog, but no contractions were apparent in the other
two dogs. Nevertheless, as illustrated in Fig. 1 (right),
the increases in blood flow to a 1-s tetanic contraction and
a train of contractions were abolished in all three dogs.

The new data, presented in Fig. 1, are virtually
identical to those presented in Fig. 5 of our original
paper (1). The findings strengthen the conclusions
from that paper and from Dyke et al. (3) that acetyl-

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**Fig. 1.** Blood flow responses to sciatic nerve stimu-
lation in 3 dogs before and after neuromuscular blockade
with α-bungarotoxin (0.2 mg/kg body wt). Data for
control conditions are the peak flows observed. Because
no changes in blood flow were discernible after α-bun-
garotoxin, data were taken at the same time that the
peak occurred under control conditions. Values are
means ± SE. Single, 1-s stimulation (30 Hz, 0.1 ms,
10 × motor threshold); train, 30-s stimulation train (30
Hz, 0.1 ms, 10 × motor threshold, 50% duty cycle).

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G. van Santen
J. M. K. H. Wierda
Department of Anesthesiology
University Hospital Groningen, Groningen, The Netherlands
E-mail: j.m.k.h.wierda@med.rug.nl

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**REPLY**

To the Editor: We thank Dr. van Santen and Dr.
Wierda for the opportunity to provide further data to
clarify the involvement of acetylcholine receptors in
the vascular responses to skeletal muscle contrac-
tion.

A muscle contraction is initiated when acetylcholine
is released from the motor nerve terminal and inter-
acts with a neuromuscular nicotinic acetylcholine re-
ceptor on the motor end plate, causing depolarization
that is propagated to the muscle fiber. The regulation
of transmitter release at neuromuscular junctions is
not fully understood and may vary depending on the
type of muscle. Blockade of contraction is traditionally
achieved by depolarizing blocking agents that may
initiate transient muscle fasciculation or nondepolar-
izing agents, which are the standard clinical drugs
employed for muscle relaxation. Vecuronium, a clini-
cally used nondepolarizing agent, was chosen for our
study (1) because of its limited cardiovascular side
effects. As pointed out by van Santen and Wierda,
nondepolarizing blockers do not interact exclusively

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choline spillover is not the physiological mechanism underlying contraction-induced vasodilation. Furthermore, because the muscle pump cannot account for the magnitude of increase in blood flow or the time course of the blood flow response observed after a 1-s contraction, our findings suggest that there is rapid release of a vasoactive substance during muscle contraction. Despite more than a century of research and the identification of a plethora of candidates, the identity of this vasoactive substance remains an enigma.

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Philip S. Clifford
Zoran Valic
Jay S. Naik
John B. Buckwalter
Departments of Anesthesiology and Physiology
Medical College of Wisconsin and Veterans Affairs Medical Center Milwaukee, Wisconsin 53295