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# Botulinum toxin abolishes sweating via impaired sweat gland responsiveness to exogenous acetylcholine

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## Summary

**Background—**Botulinum toxin A (BTX) disrupts neurotransmitter release from cholinergic nerves. The effective duration of impaired sweat secretion with BTX is longer relative to that of impaired muscle contraction, suggesting different mechanisms in these tissues.

**Objectives—**The aim of this study was to test the hypothesis that BTX is capable of altering sweating by reducing the responsiveness of the sweat gland to acetylcholine.

**Methods—**BTX was injected into the dorsal forearm skin of healthy subjects at least 3 days before subsequent assessment. On the day of the experiment, intradermal microdialysis probes were placed within the BTX-treated area and in an adjacent untreated area. Incremental doses of acetylcholine were administered through the microdialysis membranes while the sweat rate (protocol 1; $n = 8$) or a combination of sweat rate and skin blood flow (protocol 2; $n = 8$) were assessed.

**Results—**A relative absence of sweating was observed at the BTX site for both protocols (protocol 1: 0·05 ± 0·09 mg $cm^{-2}$ $min^{-1}$; protocol 2: 0·03 ± 0·04 mg $cm^{-2}$ $min^{-1}$, both at the highest dose of acetylcholine), while the sweat rate increased appropriately at the control sites (protocol 1: 0·90 ± 0·46 mg $cm^{-2}$ $min^{-1}$; protocol 2: 1·07 ± 0·67 mg $cm^{-2}$ $min^{-1}$). Cutaneous vascular conductance increased to a similar level at both the BTX and control sites.

**Conclusions—**These results demonstrate that BTX is capable of inhibiting sweat secretion by reducing the responsiveness of the sweat gland to acetylcholine, while not altering acetylcholine-mediated cutaneous vasodilatation.

### Keywords

acetylcholine; cholinergic nerve; muscarinic receptor; skin blood flow; sweat rate

Sweating is critical for thermoregulation during exercise and/or exposure to hot environmental conditions, particularly when the temperature of the environment is greater than the skin temperature.[1] Sweating is initiated upon the release of acetylcholine from sympathetic cholinergic nerves, followed by acetylcholine binding to muscarinic receptors on the sweat glands. Clinically abnormal sweating such as axillary, palmar and sole hyperhidrosis is a

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**Conflicts of interest**
None declared.

relatively common disorder and is often treated with botulinum toxin (BTX) injections.[2–4] BTX acts at cholinergic nerve terminals to block the release of acetylcholine and co-transmitters by inhibiting the fusing/docking of neurotransmitter-containing vesicles to the synaptic membrane.[5] BTX is also used for conditions such as muscle spasm disorders (hemifacial and blepharospasm), dystonia (cervical, lingual and oromandibular), strabismus and masseteric hypertrophy.[6] Interestingly, depending on the dose of BTX, the duration of effectiveness of BTX in inhibiting muscular contraction is approximately 4 months,[7] whereas its effects on inhibiting sweating can last for 6 months or longer.[8,9] Aside from the involvement of different nerves (i.e. sympathetic sudomotor C fibres vs. cholinergic α-motor neurons), the mechanisms resulting in this difference in the duration of action of BTX are not clear.

Aquaporin (AQP) 5 is a water channel protein that is essential for appropriate sweat secretion.[10,11] Interestingly, BTX inhibits water permeability in isolated rabbit cortical collecting ducts via an AQP-dependent mechanism, specifically AQP2.[12] In terms of water transport properties, AQP5 is functionally and phylogenetically similar to AQP2.[13] Given that AQP proteins have been identified in human sweat glands,[10] coupled with cholinergic denervation decreasing AQP5 expression in rat submandibular glands,[14] perhaps BTX alters sweat gland function by inhibiting water flux. Alternatively, or in addition to, chronic denervation, such as a spinal cord injury,[15] reduces sweating responses to exogenously administered sudorific agents suggesting that functional denervation of cholinergic nerves due to BTX may also disrupt sweat gland responsiveness. It may therefore be that BTX inhibits human sweating via the recognized presynaptic mechanism as well as a heretofore unrecognized mechanism that reduces the responsiveness of the sweat gland to acetylcholine. Thus, the purpose of this study was to test the hypothesis that BTX is capable of inhibiting/abolishing sweating through a mechanism other than suppression of neurotransmitter release from cholinergic nerves.

## Materials and methods

### Subjects

Fourteen healthy subjects (nine men and five women) gave their written informed consent to participate in this study. Eight subjects participated in each of two protocols, with two subjects participating in both protocols. The subjects' mean (± SD) age, height and weight were 32 (± 6) years, 174·3 (± 7·4) cm and 73·2 (± 9·2) kg, respectively. Subjects were informed of the purpose and risks of this study before providing their written consent. The protocol and informed consent were approved by the University of Texas Southwestern Medical Center at Dallas and the Presbyterian Hospital of Dallas. Subjects were instructed to refrain from alcohol and exercise 24 h before the study and caffeine 12 h before the study.

### Drug administration

BTX (Allergan Inc., Irvine, CA, U.S.A.; 10 units in 0·15 mL Ringer's solution) was administrated to dorsal forearm skin by intradermal injection at least 3 days, but no more than 10 days, prior to experimental evaluation. On the day of the experiment, confirmation of an effective block at the BTX site was evaluated by exposing the subjects to a brief whole-body heat stress via a water-perfused suit (Med-Eng Systems, Ottawa, Ontario, Canada), while skin blood flow was assessed with a laser Doppler imager (Moor LDI; Moor Instruments, Millwey, Axminster, U.K.). The absence of sweating and cutaneous vasodilatation to this heat stress at the BTX site, as previously reported,[16–18] served as validation of an effective blockade. The subjects' internal temperature was then returned to normothermic values. Two microdialysis probes were placed in the intradermal space of the dorsal forearm: one probe was placed in the area pretreated with BTX, while the other probe was placed in adjacent untreated skin. The probes were perfused with Ringer's solution (Baxter, Deerfield, IL, U.S.A.) at a rate of 2 μL $min^{-1}$ via a perfusion pump (Harvard Apparatus, Holliston, MA, U.S.A.). Approximately 90–

120 min after probe placement, once the hyperaemic response associated with the membrane placement had subsided, experimental procedures were performed as described below.

### Experimental procedure

**Protocol 1—**Sweat capsules were placed directly over the microdialysis membranes.[19] The local skin temperature, measured by a thermocouple (Type T; Omega Engineering, Stanford, CT, U.S.A.) at both sites, was increased to ~38 °C with a local heating device and maintained at this temperature throughout the experiment. After 5 min of baseline data collection, seven doses of acetylcholine ($1 \times 10^{-6}$ to 1 mol $L^{-1}$ in 10-fold increasing increments) were administered for 5 min per dose. Prior to the beginning of the each 5 min period, both probes were primed with the new concentration of acetylcholine such that the membrane portion of the probe received the new dose of the drug for at least 5 min. Sweat on the surface of the skin within the capsule evaporated into the nitrogen gas perfusing the capsule at a rate of 300 mL $min^{-1}$, while absolute humidity of the effluent nitrogen gas was continuously monitored by capacitance hygrometry (HMP233; Vaisala Inc., Woburn, WA, U.S.A.). The sweat rate was calculated from absolute humidity and the flow rate of nitrogen gas perfused through the capsule.

**Protocol 2—**Sweat chambers that have the capability to also house laser Doppler flow probes (Moor Instruments) were placed over both membranes such that the sweat rate and skin blood flow were simultaneously assessed.[20] Given the assessment of skin blood flow for this protocol, coupled with the fact that local heating increases skin blood flow, the local skin temperature at each site was clamped at a lower temperature (i.e. 34 °C) relative to protocol 1. After 5 min of baseline data collection, eight doses of acetylcholine were administered ($1 \times 10^{-7}$ to 1 mol $L^{-1}$ at 10-fold increasing increments) at 5 min per dose as outlined in protocol 1. Systolic and diastolic blood pressures were measured via auscultation of the brachial artery from the opposite arm (Tango; Suntech Medical Instruments, Raleigh, NC, U.S.A.) relative to where the sweat rate and skin blood flow were measured. Cutaneous vascular conductance was calculated from the ratio of skin blood flow to mean arterial pressure.

### Data analysis and statistics

Data were recorded at 50 Hz with a 16-bit A/D converter (Biopac, Santa Barbara, CA, U.S.A.). The sweat rate for both protocols and cutaneous vascular conductance for protocol 2 were averaged during the final 30 s of each dose of acetylcholine. These averaged values were compared between sites and doses of acetylcholine by a repeated measures two-way ANOVA (drug × site). Statistical significance was set at $P < 0{\cdot}05$. All data were expressed as mean ± SD.

## Results

The local skin temperature was maintained at 38 °C throughout protocol 1. This temperature was selected to increase the responsiveness of the sweat gland to exogenous acetylcholine. Appropriate sweating in response to exogenous acetylcholine was observed at the control site, while the increase in sweat rate at the BTX-treated site was comparatively nonexistent ($P < 0{\cdot}01$ for interactive term of the ANOVA; Fig. 1).

Sweat rate and skin blood flow were simultaneously assessed from the same location directly over the microdialysis membrane in protocol 2. For this protocol the local temperature was clamped at 34 °C. This temperature was selected to identify the full effect of acetylcholine on skin blood flow, a component of which would be masked if the local temperature was elevated to 38 °C due to local heating-induced cutaneous vasodilatation. Similar to that observed in protocol 1, the sweat rate at the BTX-treated site was absent, while appropriate sweating was

observed at the control site, resulting in significant differences in the increase in sweat rate between these sites ($P < 0{\cdot}01$ for interactive term of the ANOVA; Fig. 2, upper panel). In contrast, cutaneous vascular conductance (calculated from the ratio of skin blood flow to mean arterial pressure) increased similarly at the BTX-treated and control sites during exogenous acetylcholine administration.

## Discussion

This study clearly demonstrates that BTX has the capacity to abolish sweating via a mechanism not directly related to the suppression of neurotransmitter release from cholinergic nerves. Evidence for this is provided by the findings that exogenous acetylcholine administration did not increase the sweat rate at the BTX-treated site for either protocol, whereas appropriate sweating occurred at the control site. In contrast, cutaneous vasodilatation in response to exogenous acetylcholine was similar between the control and BTX-treated sites.

Exogenous administration of acetylcholine increases skin blood flow as well as the sweat rate.[21–23] As reduced blood flow attenuates the sweat rate,[24] in protocol 1 the local skin temperature was increased to 38 °C and clamped at this level for both sites. This was done to normalize skin blood flow between sites, although skin blood flow was not measured in that protocol. Moreover local heating improves sweating responsiveness, presumably via facilitated neurotransmitter release and augmented glandular responsiveness.[25] No sweating was observed at the BTX-treated site, while appropriate sweating occurred at the control site, suggesting that BTX treatment can inhibit human sweating by altering the responsiveness of the sweat gland to acetylcholine.

In addition to the effects of acetylcholine in causing sweat glands to secrete sweat, acetylcholine also induces cutaneous vasodilatation through the effects of nitric oxide, prostaglandins, and perhaps hyperpolarizing factors,[21,22,26–28] which are primarily mediated via a muscarinic receptor-dependent system. [16] Protocol 2 was implemented simultaneously to evaluate skin blood flow and sweat rate response to varying concentrations of acetylcholine. In contrast to that observed with sweating, the magnitude of cutaneous vasodilation in response to exogenous acetylcholine was not significantly affected at the BTX-treated site. These data suggest that the inhibition of sweating by BTX is not via a muscarinic-dependent mechanism, as inhibition of cutaneous vasodilation would have been observed at the BTX site.

The mechanism at the sweat gland by which BTX inhibits sweating is unknown, but can be speculated upon. Acetylcholine released from sympathetic cholinergic nerves binds to muscarinic receptors, presumably present in the basolateral membrane of the eccrine gland clear and dark cells, and drives secretion of sweat through a Na–K–2Cl co-transporter system.[29,30] Nejsum *et al.*[11] revealed the presence of water channel AQP proteins in rat and mouse sweat glands. In that study, pilocarpine-induced sweat secretion was reduced in AQP5-null mice relative to wild-type mice, suggesting that AQP5 has a role in fluid supply to sweat glands. AQP5 immunoreactivity was also observed at the dark cells of the secretory portion of human eccrine sweat glands.[10] The primary action of BTX is the degradation of SNAP-25 (synaptosome-associated protein of 25 kDa), a SNARE [soluble NSF (*N*-ethylmaleimide-sensitive fusion protein) attachment protein receptor] protein required for the docking and fusing of neurotransmitter-containing vesicles.[5] SNAP-25 is colocalized to vesicles containing AQP2 in rat kidney,[31] and its water permeability can be inhibited by BTX.[12] Given these observations, perhaps SNARE proteins are also in AQP5 of human sweat glands, although we are unaware of studies that have investigated this hypothesis. If such SNARE proteins are present in AQP5 of human sweat glands, then perhaps disruption of water flux through AQP channels is the mechanism by which BTX inhibits sweating at the level of the sweat gland.

On the other hand, cholinergic denervation decreases AQP5 expression in the rat submandibular glands, intraglandular injection of BTX reduces salivary secretion due in part to glandular atrophy, and surgical sympathectomy or spinal cord transection decreases sweat gland sensitivity.[14,15,32,33] Thus, as other possibilities, the inhibition of AQP5 expression, atrophy of the sweat gland, and/or reduced sweat gland sensitivity to cholinergic stimulation secondary to disruption of neuronal transmission following BTX administration may contribute to the responses shown in Figures 1 and 2, although it remains unknown whether < 10 days (e.g. as few as 3 days in most subjects) of functional cholinergic denervation is sufficient to cause these responses in humans. Furthermore, in the aforementioned studies, responses were suppressed or inhibited [14,32,33] as opposed to the response being completely abolished in the present study (Figs 1 and 2).

In conclusion, in addition to the recognized mechanism by which BTX suppresses sweating (i.e. inhibition of neurotransmitter release from cholinergic nerves), the present data clearly demonstrate that BTX is also capable of abolishing sweating caused by exogenous acetylcholine and thus can disrupt sweating by reducing the responsiveness of the sweat gland to acetylcholine. Interestingly BTX did not alter cutaneous vasodilator responsiveness to acetylcholine. It remains unknown whether the observed effects of BTX on sweating are secondary to functional denervation of the cholinergic nerve and/or are due to an effect of BTX acting directly on the sweat gland to alter water flux. Thus, future studies are required to identify the precise mechanism(s) by which BTX reduces the responsiveness of sweat glands to acetylcholine.

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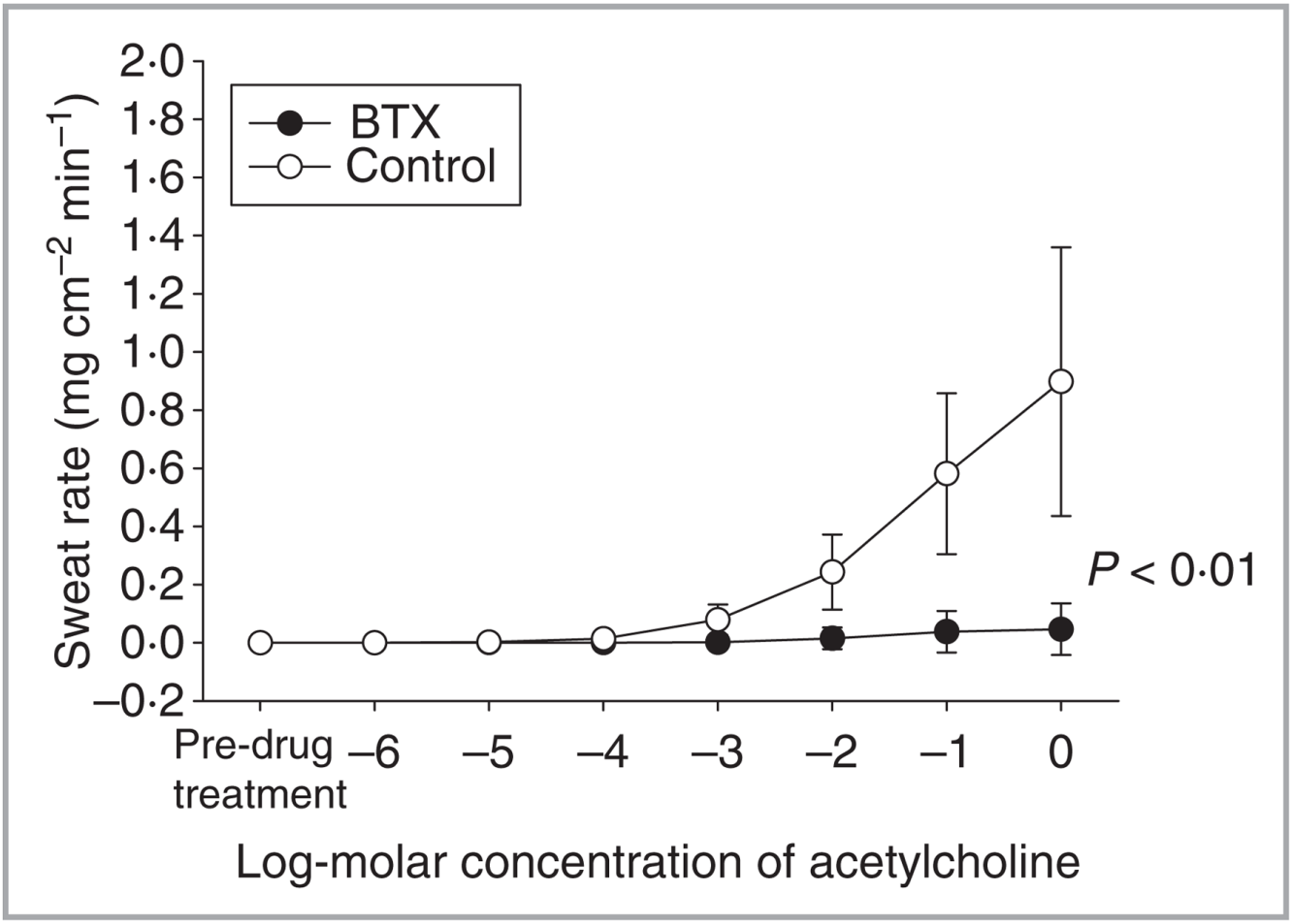


**Fig 1.**

Sweat rate at botulinum toxin (BTX)-treated (black circle) and control (open circle) sites during multiple administrations of acetylcholine (ranging from $1 \times 10^{-6}$ to 1 mol $L^{-1}$) for protocol 1. The indicated *P*-value is from the interactive effect of the two-way repeated measures ANOVA. The large variance (standard deviation) around mean sweating is due to variation in sweat rate between subjects for the indicated dose of acetylcholine, not due to within-subject variation. Nevertheless, the increase in sweat rate at the BTX site was relatively absent when compared with the control site.

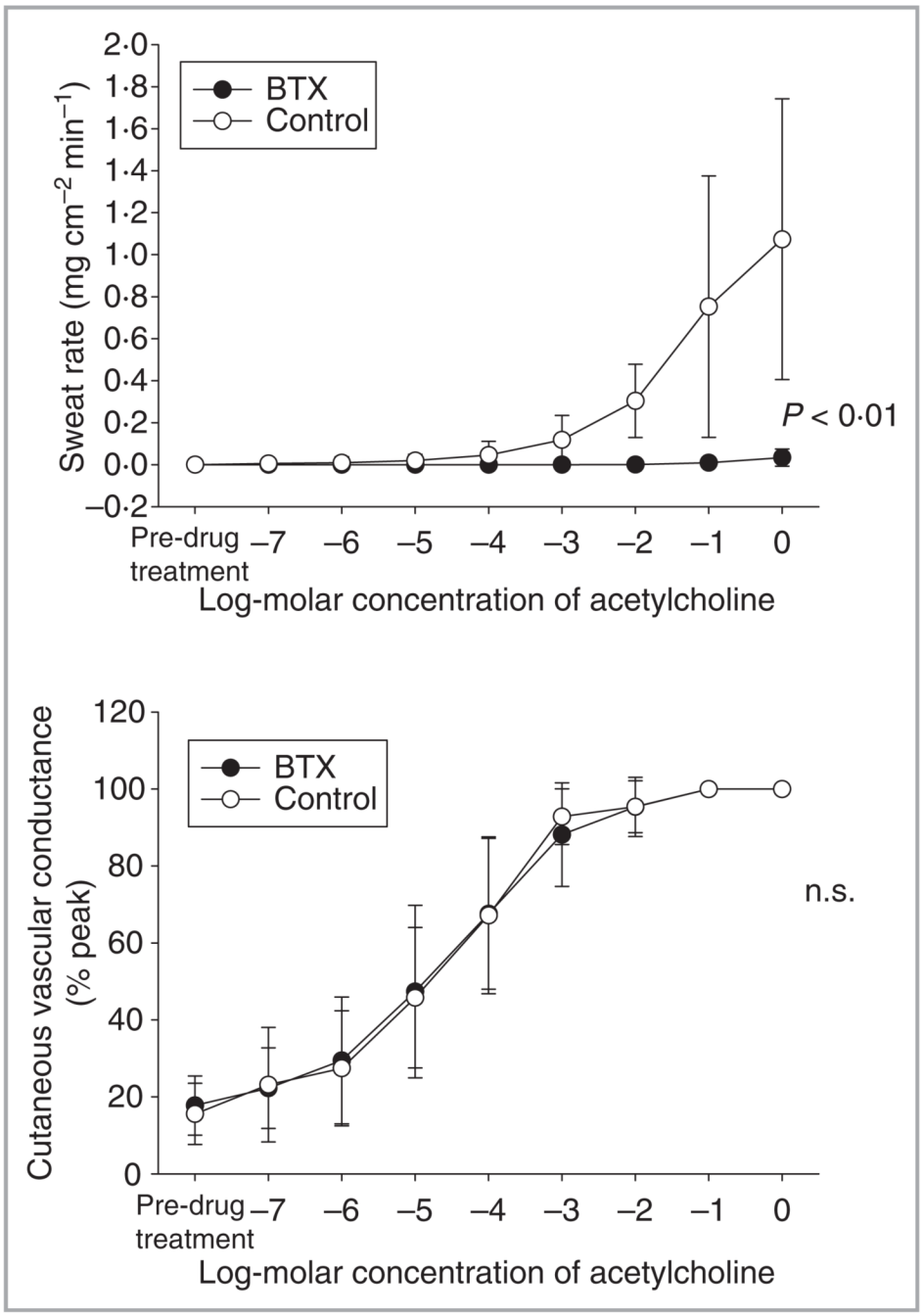


**Fig 2.**

Sweat rate (upper panel) and cutaneous vascular conductance (CVC, lower panel) at botulinum toxin (BTX)-treated (black circle) and control (open circle) sites during multiple administrations of acetylcholine (ranging from $1 \times 10^{-7}$ to 1 mol $L^{-1}$) for protocol 2. CVC was normalized to peak vasodilatation values measured during administration of high concentrations of acetylcholine. The data are expressed as a percentage of that peak response. Importantly, CVC expressed in absolute units (i.e. non-normalized CVC) was not affected by BTX administration (BTX: 297·7 ± 117·5; control: 290·0 ± 119·9 arbitrary units per mmHg). The indicated *P*-value is from the interactive effect of the two-way repeated measures ANOVA.