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The Effect of Guanethidine and Local Anesthetics on the Electrically Stimulated Mouse Vas Deferens

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Complex regional pain syndrome is often treated with the sympatholytic guanethidine and a local anesthetic in a Bier's block. The efficacy of this treatment has been questioned. Because local anesthetics inhibit the norepinephrine uptake transporter, we hypothesized that this variable efficacy results from the local inhibiting the uptake of guanethidine. In this study, we tested this hypothesis by using a sympathetically innervated mouse vas deferens preparation. Organ bath-mounted mouse vasa deferentia were electrically stimulated in the absence and presence of guanethidine, prilocaine, procaine, and cocaine in various combinations. Prilocaine (1 mM) induced an immediate inhibition of twitch response (maximum 100% after 2 min) that fully reversed after washing. Guanethidine (3 μM) also inhibited twitching by 95% ± 3% in 15 min, but this effect was only partially reversed after 1 h of washing (33% ± 12% of control). When prilocaine and guanethidine were added in combination, a reversal of 80% ± 13% (at 1 h) was observed. Procaine (300 μM) produced a transient increase (152% ± 14%) in response. When co-incubated with guanethidine (3 μM), the twitch was reduced to 24% ± 4% of control and was reversed to 77% ± 7% after 1 h. Cocaine (30 μM) inhibited the twitch response to 53% ± 8%, which was fully reversed by 1 h of washing. When co-incubated with guanethidine, the response was reduced to 39% ± 6% of control and was reversed to 86% ± 10% after 1 h. In all cases, the reversal produced by the combination was significantly more intense ($P < 0.05$) than that produced by guanethidine alone. Local anesthetics reduce the sympatholytic actions of guanethidine, and this may explain the variable efficacy of guanethidine in the treatment of complex regional pain syndrome.

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Complex regional pain syndrome is a chronic pain syndrome characterized by dysfunctional sympathetic activity (1). Current treatment regimens for this condition include surgical or pharmacological sympathectomy (2–5). Pharmacological sympathectomy involves blocking the corresponding sympathetic nerves with specific drugs or regional anesthesia techniques. IV regional guanethidine Bier's block (IVRGBB), which was first described in 1974 (2), is used often. Guanethidine is a substrate for the norepinephrine (NE) transporter (NET), affording specificity for sympathetic nerves. Once taken up by the NET, guanethidine displaces NE from the cell's releasable stores, providing a chemical sympathectomy (6). Guanethidine and a local anesthetic, such as prilocaine, are injected IV; the local anesthetic provides relief from the discomfort produced by guanethidine. The inclusion of a local anesthetic also allows manipulation of the affected extremity beyond the range normally tolerated by the patient (7).

The treatment of complex regional pain syndrome with IVRGBB has been reported to have variable efficacy (8). Because the local anesthetic, cocaine, competitively inhibits the uptake of NE (9), we hypothesized that other local anesthetics may interact with the NET to modify uptake. Indeed, we have previously demonstrated significant inhibition of uptake with clinical concentrations of a range of local anesthetics, including prilocaine (10,11).[1] We suggest that the variable efficacy of IVRGBB results from the local anesthetic

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[1] Joyce PI, Atcheson R, Rowbotham DJ, et al. Prilocaine and lidocaine inhibit [^{3}H]noradrenaline uptake into 293-hNET cells [abstract]. Br J Anaesth 1998;80(Suppl 1):A432.

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reducing the uptake of guanethidine, resulting in a partial, short-lived sympathectomy.

The primary aim of this study was to test the hypothesis that local anesthetics impair the uptake of guanethidine in the mouse vas deferens (mVD), a sympathetically innervated *ex vivo* preparation. Specifically, our goals were the following.

1. To examine the effects of guanethidine (a sympathomimetic) and local anesthetics alone.
2. To determine whether local anesthetics (amide; prilocaine, ester; procaine; and the known NET inhibitor, cocaine) modify the actions of guanethidine.

Methods

This study was approved by the animal use committee of the University of Ferrara, Ferrara, Italy. Vasa deferentia were taken from male Swiss mice (25–30 g), according to Calo' et al. (12). The tissues were suspended in 5-mL organ baths containing Krebs solution (Mg^{2+} free; 2.5 mM $CaCl_2$; pH 7.4) bubbled with 95% oxygen and 5% CO_2 at 33°C, and a resting tension of 0.3 g was applied.

The mVDs were continuously stimulated by two electrodes with supramaximal voltage (30-V) rectangular pulses of 1-ms duration and 0.05-Hz frequency. Electrically evoked contractions were measured isotonically with a strain gauge transducer (Basile 7006; Comerio VA, Italy), and responses were recorded with a Basile recorder (Model Gemini 7070; Comerio VA). After an equilibration period of ~60 min, the contractions induced by electrical field stimulation (EFS) were stable. In the initial series of experiments, tetrodotoxin (0.3 μM) and prazosin (1 μM) were added to the baths to confirm the neurogenic nature of the contraction and the involvement of α_1 adrenoceptors. Prilocaine 1 mM, procaine 300 μM, and cocaine 30 μM (estimated from binding studies) (11) were added for 15 min and then washed at 15-min intervals for up to 75 min to determine any direct effects and reversibility. In other experiments, guanethidine (3 and 10 μM) was added for 15 min and then washed at 15-min intervals for up to 75 min. This extensive washing procedure was used to ensure removal of local anesthetic and guanethidine. Finally, local anesthetic and guanethidine combinations (prilocaine 1 mM plus guanethidine 3 and 10 μM; procaine 300 μM plus guanethidine 3 μM; and cocaine 30 μM plus guanethidine 3 μM) were tested in the same manner. A time control (no added drug) was also included in each experiment.

Data are presented as the mean percentage of control (baseline) EFS-induced twitch (±SEM) values for n individual experiments, as described in the figure legends. Statistical comparisons were made by Student's t-test and analysis of variance, as appropriate, and differences were considered significant when $P < 0.05$

Results

EFS elicited a twitch response that returned to baseline in <2 s. After a period of washing and stabilizing (approximately 60 min), a twitch response relatively constant in amplitude was achieved. This control (baseline) state continued for >75 min. EFS contraction with the variables used (30 V, 1 ms, 0.05 Hz) was clearly neurogenic and mediated by α_1 adrenoceptors, because the contraction was fully blocked by tetrodotoxin (0.3 μM) and partially reversed by prazosin (1 μM) (Fig. 1).

Prilocaine at 1 mM induced an immediate inhibition of twitch response that reached a maximum (100%) after 2 min. This inhibitory effect was rapidly reversible after washing. Guanethidine (3 μM) also inhibited baseline EFS twitch by 95% ± 3% in 15 min (Fig. 2). However, in contrast to prilocaine, this effect was only partially reversed after 1 h of washing (33% ± 12% of control values). When prilocaine and guanethidine were added in combination at 1 mM and 3 μM, respectively (Fig. 2), a reversal of 80% ± 13% was observed after 1 h, which was significantly greater ($P <$ 0.05) than the reversal produced by guanethidine (3 μM) alone. These reversal data are summarized in Figure 3.

Increasing the guanethidine concentration to 10 μM inhibited the EFS twitch response to 4% ± 3% of control, and it was reversed to 8% ± 3% after 1 h of washing. When prilocaine (1 mM) was co-incubated with guanethidine (10 μM), the EFS twitch was inhibited to 2% ± 2% of control but reversed to 30% ± 10% after 1 h of washing (Fig. 4). The reversal produced by the combination was significantly greater ($P < 0.05$) than that produced by guanethidine alone and is suggestive of a competitive interaction between guanethidine and prilocaine.

Procaine (300 μM) alone facilitated the twitch response to 152% ± 14% of control but returned to control levels 10 min after the first wash, from which point the trace remained stable. When co-incubated with guanethidine (3 μM), the EFS twitch was reduced to 24% ± 4% of control and reversed to 77% ± 7% of control after 1 h of washing. The reversal produced by the combination was significantly greater ($P < 0.05$) than that produced by guanethidine alone (Fig. 5). Reversal data are summarized in Figure 3.

Cocaine (30 μM) inhibited the EFS twitch response to 53% ± 8% of control and was completely reversed by 1 h of washing. When co-incubated with guanethidine (3 μM), the EFS twitch was reduced to 39% ± 6% of control and was reversed to 86% ± 10% after 1 h of washing. The reversal produced by the combination was significantly greater ($P < 0.05$) than that produced by guanethidine alone (Fig. 6). Reversal data are summarized in Figure 3.

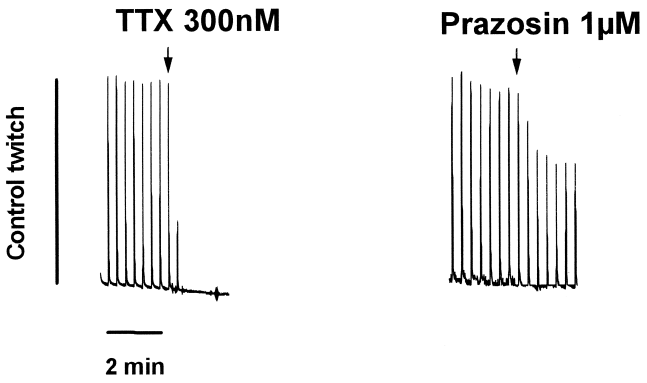

Figure 1. Effects of 300 nM tetrodotoxin (TTX) and 1 μM prazosin on the electrical field stimulated contraction of the isolated mouse vas deferens. Data are from a single representative experiment and also illustrate the magnitude and stability of the measured contractile response.

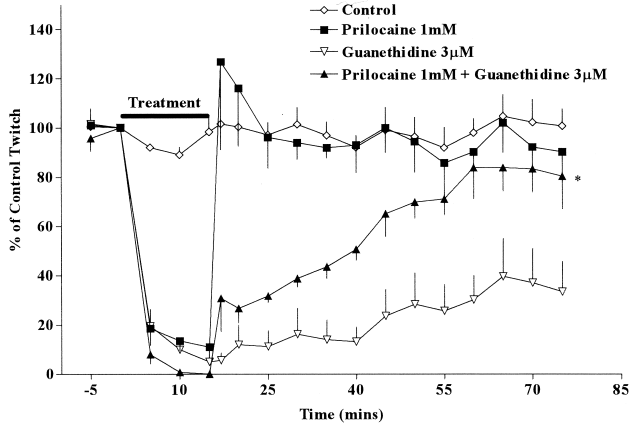

Figure 2. The effects of prilocaine 1 mM, guanethidine 3 μM, and prilocaine plus guanethidine on the electrical field stimulated contraction of the isolated mouse vas deferens. Data are mean ± SEM for $n \geq 4$. *Whole prilocaine and prilocaine plus guanethidine curves were different ($P < 0.05$) by analysis of variance.

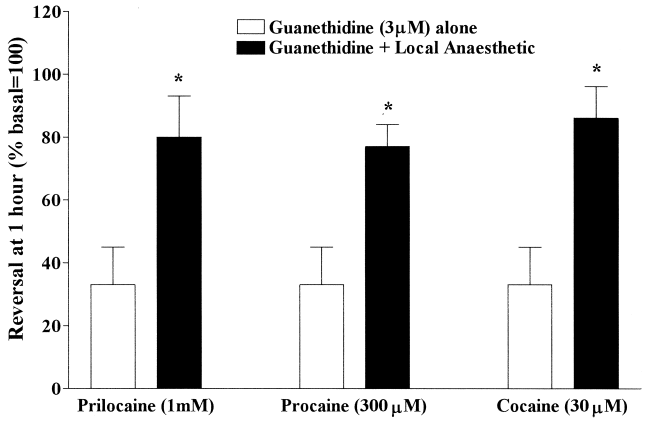

Figure 3. Comparison of the reversal at 1 h for guanethidine 3 μM alone (open bar) or in the presence of prilocaine 1 mM, procaine 300 μM, or cocaine 30 μM (black bar). Data are mean ± SEM for $n \geq 4$. *$P < 0.05$; significantly different compared with guanethidine alone.

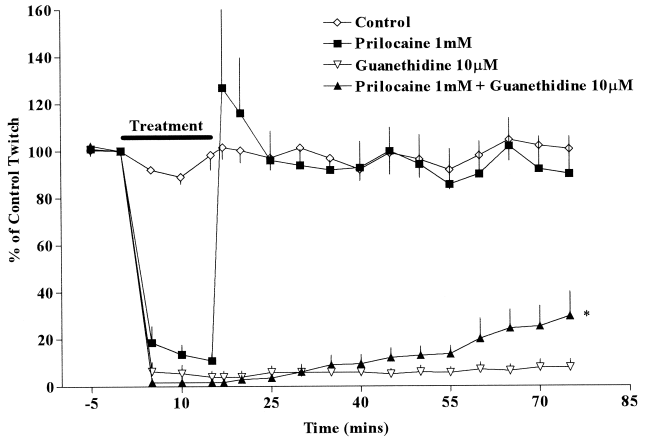

Figure 4. The effects of prilocaine 1 mM, guanethidine 10 μM, and prilocaine plus guanethidine on the electrical field stimulated contraction of the isolated mouse vas deferens. Data are mean ± SEM for $n \geq 4$. *Whole prilocaine and prilocaine plus guanethidine curves were different ($P < 0.05$) by analysis of variance.

Discussion

We have clearly demonstrated that prilocaine and cocaine inhibit EFS twitches of the mVD in a reversible manner. In contrast, procaine produced a small, transient enhancement of twitches. Guanethidine, as expected, also reduced this response, confirming its sympatholytic effect. However, the most important finding of this study was that, when co-administered (as would occur in IVRGBB), the local anesthetic reduced the effect of guanethidine and that this could be partially overcome by increasing the concentration of guanethidine.

These data confirm and extend our previous study in SH-SY5Y human neuroblastoma cells as a model of human sympathetic neurons *in vitro* (11). In this study, we demonstrated that a range of local anesthetics (including prilocaine, procaine, and cocaine) and unlabeled guanethidine (unpublished data) inhibited the uptake of [^{3}H]NE, which we used as a surrogate for [^{3}H]guanethidine (which is commercially unavailable), in a concentration-dependent manner. In addition, these same local anesthetics and unlabeled guanethidine (unpublished data) also inhibited the binding of the selective NET radiolabel [^{3}H]nisoxetine (13,14). There was a positive correlation between the inhibition of uptake and binding ($r^2 = 0.68$; $P < 0.05$). Moreover, we have similar data in HEK293 cells expressing the recombinant human NET (10) (unpublished data).[1] These data suggest that local anesthetics may inhibit the uptake of guanethidine and form the rationale for performing the present investigation in the mVD. In our previous studies, there was a competitive inhibition of [^{3}H]NE uptake and displacement of [^{3}H]nisoxetine binding that we wished to probe further *ex vivo*.

When 1 mM prilocaine and 3 μM guanethidine were co-administered, the reversal produced by this

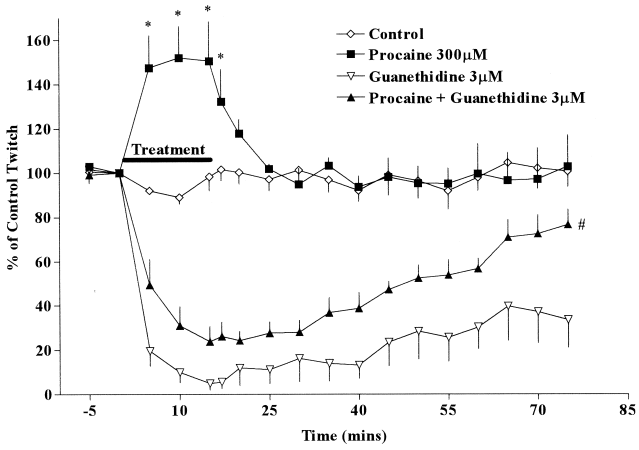

Figure 5. The effects of procaine 300 μM, guanethidine 3 μM, and procaine plus guanethidine on the electrical field stimulated contraction of the isolated mouse vas deferens. Data are mean ± SEM for $n \geq 4$. #Whole procaine and procaine plus guanethidine curves were different ($P < 0.05$) by analysis of variance. *Increased compared with control.

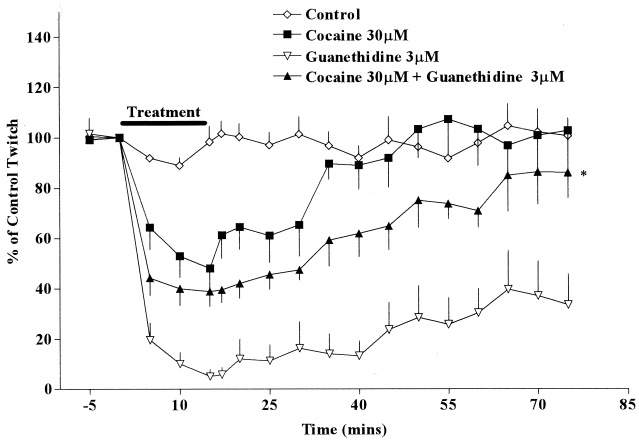

Figure 6. The effects of cocaine 30 μM, guanethidine 3 μM, and cocaine plus guanethidine on the electrical field stimulated contraction of the isolated mouse vas deferens. Data are mean ± SEM for $n \geq 4$. *Whole cocaine and cocaine plus guanethidine curves were different ($P < 0.05$) by analysis of variance.

combination after one hour of washing was 80% (compared with 33% for guanethidine alone). However, when the guanethidine concentration in the bath was increased to 10 μM, the reversal was reduced to 30% (compared with 8% for guanethidine alone). These data are suggestive of a competitive interaction in that the reduction of the sympatholytic activity of guanethidine produced by prilocaine could be overcome by increasing the guanethidine (NET substrate) concentration. Competitivity for procaine and cocaine were not formally examined in this investigation, but we have no reason to suspect any other mode of interaction. Despite a presumed competitive interaction, increasing the guanethidine dose in humans would be unwise because of its poor side-effect profile.

While the amide prilocaine is the most commonly used local anesthetic in IVRGBB (8), we wanted to test a representative ester-type local anesthetic (procaine) and a known inhibitor of NET (cocaine). The inhibition of NET activity by cocaine is used in nasal surgery to produce vasoconstriction and hence reduce bleeding (15). The observation that procaine and cocaine inhibited the actions of guanethidine adds weight to our working hypothesis that local anesthetics inhibit the uptake of guanethidine.

The degree of twitch depression produced by 30 μM cocaine alone was only ~50%, which is in marked contrast to the inhibition produced by prilocaine (>70%). It is unlikely that the initial reduction in contraction in response to local anesthetic application is due to NET inhibition. It is more likely that this results from interference with neuronal voltage-sensitive channels (e.g., Na^+), and cocaine at 30 μM may produce a partial inhibition of Na^+ channels. This local anesthetic produced a smaller twitch inhibition than prilocaine, but as we have previously demonstrated, it produces a full inhibition of NET activity (11).[1]

It is worthy of mention that procaine produced a small, transient increase in twitch amplitude. The mechanism for this increase in unclear. It is possible that the increase in twitch amplitude may be due to inhibition of guanosine triphosphatase activity within the postsynaptic cell. Hageluken et al. (16) reported that procaine, at the concentration used here, inhibited guanosine triphosphate (GTP) hydrolysis in cyclic adenosine monophosphate -differentiated HL-60 cells. This could potentiate the effects of G_q type G protein-coupled receptors, such as α_1 adrenoceptors, by reducing GTP hydrolysis. Interestingly, in this study, tetracaine, bupivacaine, and lidocaine were reported to increase GTP hydrolysis (16).

In summary, we have clearly demonstrated that local anesthetics inhibit the uptake of guanethidine and suggest that their coadministration in IVRGBB might explain the inconsistent clinical reports with respect to efficacy. Clinical studies are required to compare the IVRGBB in the absence and presence of local anesthetic, or traditional IVRGBB could be compared with guanethidine in sedated or anesthetized patients.

References

1. Bonica JJ. Causalgia and other reflex sympathetic dystrophies. In: Bonica JJ, ed. The management of pain. 2nd ed. Philadelphia: Lea & Febiger, 1990:220–43.
2. Hanningon-Kiff JG. Intravenous regional sympathetic block with guanethidine. Lancet 1974;1:1019–20.
3. Hanningon-Kiff JG. Relief of Sudeck's atrophy by regional intravenous guanethidine. Lancet 1977;1:1132–3.
4. Hanningon-Kiff JG. Relief of causalgia in limbs by regional intravenous guanethidine. BMJ 1979;2:367–8.

5. Hanningon-Kiff JG. Hyperadrenergic-effected limb causalgia: relief by pharmacologic norepinephrine blockade. Am Heart J 1982;103:152–3.
6. Maxwell RA. Guanethidine after twenty years: a pharmacologist's perspective. Br J Pharmacol 1982;13:35–44.
7. Gordon N. Reflex sympathetic dystrophy [review]. Brain Dev 1996;18:257–62.
8. Kingery WS. A critical review of controlled clinical trials for peripheral neuropathic pain and complex regional pain syndromes. Pain 1997;73:123–39.
9. Sora I, Hall FS, Andrews AM, et al. Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. Proc Natl Acad Sci U S A 2001;98:5300–5.
10. Joyce PI, Rowbotham DJ, Blakely RD, Lambert DG. Effects of local anaesthetic agents on [^{3}H]nisoxetine binding to recombinant human norepinephrine transporters. Br J Pharmacol 1999; 128:178P.
11. Joyce PI, Atcheson R, Marcus RJ, et al. Interaction of local anaesthetic agents with the endogenous norepinephrine transporter in SH-SY5Y human neuroblastoma cells. Neurosci Lett 2001;305:161–4.
12. Calo' G, Rizzi A, Bodin M, et al. Pharmacological characterization of nociceptin receptor: an in vitro study. Can J Physiol Pharmacol 1997;75:713–8.
13. Tejani-Butt SM. [^{3}H]Nisoxetine: a radioligand for quantitation of norepinephrine uptake sites by autoradiography or by homogenate binding. J Pharmacol Exp Ther 1992;260:427–36.
14. Tejani-Butt SM, Brunswick DJ, Frazer A. [^{3}H]Nisoxetine: a new radioligand for norepinephrine uptake sites in brain. Eur J Pharmacol 1990;191:239–43.
15. Albers FW. The clinical use of cocaine in rhinosurgery: a case-report and a review. Rhinology 1990;1:55–9.
16. Hageluken A, Grunbaum L, Nurnburg B, et. al., Lipophilic β-adrenoceptor antagonists and local anesthetics are effective direct activators of G-proteins. Biochem Pharmacol 1994;47: 1789–95.