

Cardiopulmonary Effects of the Novel Neuromuscular Blocking Drug GW280430A (AV430A) in Dogs

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Background: **This investigation determined the cardiopulmonary side effects of a novel nondepolarizing neuromuscular blocking drug with an ultrashort duration of action in anesthetized male beagles.**

Methods: **The ED_{95} for GW280430A was first determined in four animals. These data were then used to guide bolus dosing in multiples of ED_{95} in six dogs instrumented for hemodynamic measurements as well as inspiratory pressure and pulmonary compliance. Cardiopulmonary data were compared before and after the conclusion of a 60- to 90-min GW280430A infusion and in response to subsequent incremental bolus dosing starting with 3.125 × ED_{95}. An adverse response was regarded as an alteration of 10% or greater in any variable. Arterial blood was obtained for histamine analysis before and 1 min after each dose.**

Results: **The ED_{95} of GW280430A was 0.064 ± 0.01 mg/kg, and stable neuromuscular blockade was maintained with infusion of 0.012 ± 0.002 mg · kg^{-1} · min^{-1}. With the exception of a late 14% increase in heart rate, there were no cardiopulmonary changes during infusion. Bolus dosing produced no cardiopulmonary change until a decrease in mean arterial pressure was elicited in four of six dogs at 25 × ED_{95}. This response was modest, transient, and associated with a concomitant increase in plasma histamine concentration. There were no accompanying changes indicative of direct myocardial depression, pulmonary vasoconstriction, or bronchospasm.**

Conclusions: **These data indicate that GW280430 does not produce demonstrable cardiovascular effects in the anesthetized dog until doses far in excess of the ED_{95} are administered as a bolus.**

DESPITE a propensity for adverse side effects, succinylcholine remains the only rapid-onset, ultrashort-duration muscle relaxant available for clinical use. To date, dedicated efforts to develop a nondepolarizing substitute for succinylcholine have not been successful, with most compounds introduced into the marketplace exhibiting an onset of action that is too slow, a duration of action that is too long, or a profile of cardiopulmonary side effects that limit clinical utility.[1–3] Preliminary data indicate that the asymmetric mixed-onium chlorofumarate compound GW280430A (AV430A) is a nondepolarizing neuromuscular blocking drug with an ultrashort duration of action.[4,5] The current study was performed in dogs to determine the cardiopulmonary side effects of GW280430A administered as a continuous infusion or in escalating bolus doses.

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Materials and Methods

Preparation

Ten adult male beagles weighing between 10.2 and 14.3 kg were used for the study after approval of the protocol by the Institutional Animal Care and Use Committee (Weill Medical College of Cornell University, New York, New York). After an overnight fast, anesthesia was induced with 20 mg/kg intravenous thiopental, the trachea was intubated, and the lungs were ventilated with a mixture of 30% oxygen, 70% nitrous oxide, and 0.5–1.5% isoflurane. Inspiratory pressures, volumes, and flow rates were continuously monitored *via* side-stream spirometry, and inspired/expired gas composition was assessed with infrared analysis (Datex Ultima, Helsinki, Finland). From ventilatory pressures and volumes, pulmonary compliance was calculated on a breath-to-breath basis (Datex Ultima). Minute ventilation was adjusted to maintain an end-tidal partial pressure of carbon dioxide (P_{CO_2}) of approximately 30 mmHg, and body temperature was maintained at approximately 37.5°C with a water-circulating heating blanket. After placement of electrocardiographic leads, the right femoral artery was cannulated with a vascular introducer sheath for measurement of systemic arterial pressure. A superficial segment of tendon attached to the left tibialis anterior muscle was then accessed *via* a 1.0-cm incision, and a tendon loop was created with fine silk ligatures. The tendon loop was attached to a force transducer (FT10 C; Grass Instruments, Quincy, MA), preloaded with 50 g tension, and twitch responses elicited at 0.15 Hz supramaximal electrical stimulation of the peroneal nerve were recorded. Animals were assigned to two groups. In group 1 (n = 4), no further instrumentation was placed, and the ED_{95} for GW280430A was estimated (see be-

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low). In addition, the response to various infusion rates was determined in a subset of animals. These data were then used to guide bolus dosing (in multiples of ED_{95}) and continuous infusion rates (in $mg \cdot kg^{-1} \cdot min^{-1}$) in subsequent experiments. In group 2 (n = 6), a 5.0-French pulmonary artery (PA) catheter was advanced into the PA *via* the right external jugular vein, and a small left thoracotomy was performed at the fourth intercostal space to allow for placement of an electromagnetic flow probe (Carolina Medical Instruments, King, SC) around the ascending aorta. In addition, a 5-French conductance/micromanometer catheter (Millar Medical Instruments, Dallas, TX) was advanced into the left ventricle (LV) *via* the femoral arterial sheath and was used to measure volume and pressure within the chamber. The method by which the conductance catheter measures LV volume has recently been reviewed.[6] Fundamentally, the technique involves continuous measurement of the impedance to an electrical current within the heart that is proportional to volume. Offset of the volume signal due to loss of the current to surrounding tissues, or *parallel conductance*, was determined by injection of 5 ml saline, 3%, *via* the PA catheter, and stroke volume measurements were cross-calibrated with the electromagnetic flow probe. For all animals in both groups, estimated fluid deficits were replaced with lactated Ringer's solution, which was subsequently maintained at a rate of 3–4 $ml \cdot kg^{-1} \cdot h^{-1}$ throughout the experiment.

Study Design and Data Acquisition

After surgical preparation, a 15- to 20-min stabilization period was allowed before initiating the protocol. During this time, end-tidal isoflurane concentration was allowed to equilibrate at approximately 1.0%, and consistency of twitch height was confirmed. In group 1, potency of GW280430A was first assessed by bolus administration of incremental doses staring at 0.01 mg/kg until 100% block was achieved. Thirty minutes was allowed between doses, with full recovery of twitch height and verification of normal train-of-four stimulation. After initial dose–response assessment, the infusion dose required to produce 90–95% neuromuscular blockade was determined in two dogs and was found to be approximately 0.010 $mg \cdot kg^{-1} \cdot min^{-1}$. In group 2, GW280430A was first administered as a bolus of two times the ED_{95} determined in group 1. When there was approximately 90% twitch recovery, a continuous infusion of 0.010 $mg \cdot kg^{-1} \cdot min^{-1}$ was initiated. The infusion rate was then titrated to establish a 90–95% block of twitch. When stable block was achieved, the infusion was continued for 60–90 min and then discontinued. After a 30-min stabilization period, a normal response to train-of-four stimulation was verified, and the cardiopulmonary side effects of GW280430A were then determined by injection of incrementally larger bolus doses at 12-min intervals, starting with 0.2 mg/kg. With this dosing regimen, an adverse response for each animal was regarded as an alteration of 10% or greater in any of the observed cardiopulmonary variables. For this stage of the experiment, arterial blood samples were obtained for histamine analysis (immunoassay kit; Immunotech International, Marseille, France) before and 1 min after each dose, regardless of whether hemodynamic effects were evident. Hemodynamic data were recorded in both analog and digital format. From direct measurements, the first derivative of LV pressure (dP/dt) was determined along with proximal aortic blood acceleration (dQ/dt), a nonspecific beat-to-beat index of ventricular–vascular interaction, and the maximal rate of change in LV volume (dV/dt), a beat-to-beat index of LV diastolic function. In addition, beat-to-beat LV hydraulic work was calculated as the instantaneous product of LV pressure and proximal aortic blood flow with work per unit of time used as an index of LV power (PWR). The ratio of maximal PWR (PWR_{max}) to square end-diastolic volume (EDV^2) was then used as a load-insensitive index of contractility.[7]

Statistics and Data Analysis

Individual log-probit dose–response relations were assessed from a minimum of three points to determine the ED_{95} for each dog. Individual log-probit dose–time to onset (seconds from injection to maximal response) and dose–duration (minutes from injection to 95% recovery of twitch height) curves were also generated from a minimum of six points. From these curves, the time to onset of and recovery from neuromuscular blockade at multiples of the ED_{95} were determined. These values, along with dose-related alterations in cardiovascular and respiratory variables and plasma histamine concentration, were assessed by analysis of variance for repeated measures and the Newman-Keuls test where applicable. For all analyses, a P value of 0.05 or less was considered significant. All data are presented as mean ± SE.

Results

Potency, Onset, and Duration

Determination of the dose–response relation and calculation of ED_{95} were facilitated by a very short duration of action (fig. 1). In individual animals, ED_{95} ranged from 0.049 to 0.082 mg/kg, with a mean of 0.064 ± 0.008 mg/kg. At ED_{95}, onset of neuromuscular blockade ranged from 90 to 128 s (mean, 107 ± 9 s), with a duration of 3.2–6.2 min (mean, 5.2 ± 0.7 min). At $3 \times ED_{95}$, onset ranged from 44 to 74 s (mean, 58 ± 6 s; $P < 0.05$ *vs.* ED_{95}), with a duration of 4.7–8.5 min (mean, 7.0 ± 0.8 min; $P < 0.05$ *vs.* ED_{95}).

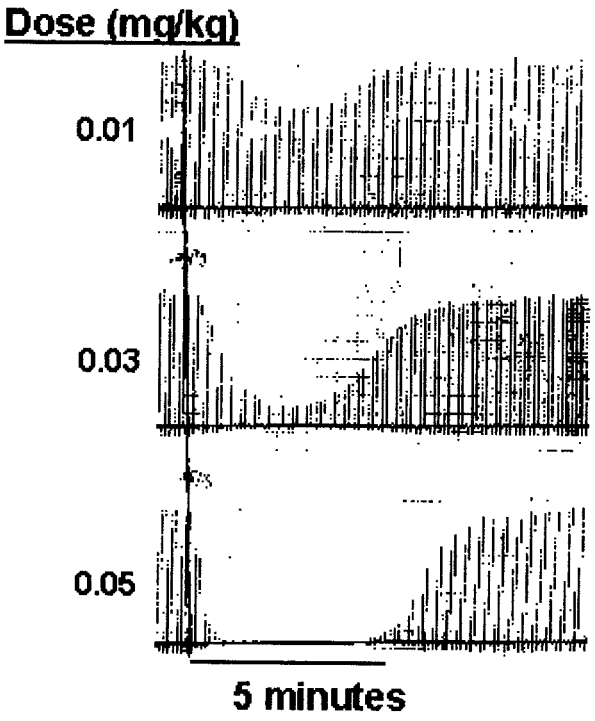

Fig. 1. Representative tibialis anterior twitch tracings in response to increasing doses of GW280430A. Responses from each animal were subjected to log-probit analysis to determine individual ED_{95}s.

Response to Continuous Infusion

Infusion rates required to produce 90–95% neuromuscular block ranged from 0.009 to 0.015 mg · kg^{-1} · min^{-1} (mean, 0.012 ± 0.002 mg · kg^{-1} · min^{-1}). A representative tracing of single-twitch height after cessation of GW280430A infusion is shown in figure 2. In the two dogs that received a 60-min infusion, single-twitch height returned to baseline after 5.1 and 3.9 min, respectively. In dogs receiving a 90-min infusion, single-twitch height returned to baseline in 3.2 ± 0.3 min. There were no changes in peak inspiratory pressure or pulmonary compliance during the infusion. The only cardiovascular change was a modest increase in heart rate from 138 ± 6 to 157 ± 6 beats/min.

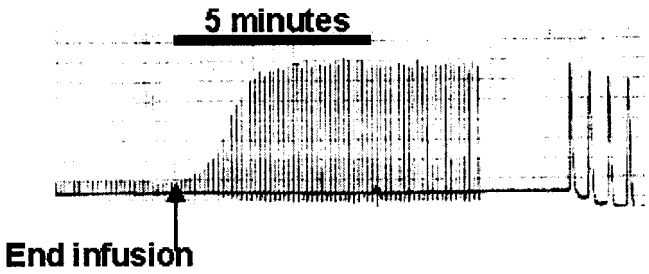

Fig. 2. Representative tracing of the rapid recovery of tibialis anterior twitch after cessation of a 90-min infusion of GW280430A at a dose sufficient to maintain 90–95% blockade.

Cardiopulmonary Side Effects and Histamine Release after Bolus Dosing

Throughout the incremental bolus dosing protocol, end-tidal carbon dioxide and isoflurane concentration as well as body temperature remained constant. As shown in figure 3, within individual animals, 25 × ED_{95} produced a decrease of 10% or greater in mean arterial pressure (MAP) (predefined as the threshold for an adverse event) in four of six dogs. Figure 4 depicts the absolute values for heart rate, pressure variables, aortic flow data, and ventilatory variables before (predose) and 1 min after (peak response) bolus injection of GW280430A. There were no differences between any of the predose baseline values and the value for each variable before initiating the bolus dosing protocol. In contrast to the relative change in MAP produced in four of six individual dogs, when the dose–response relation for each hemodynamic variable was analyzed in terms of the mean values from all animals, GW280430A produced significant hemodynamic change (limited to reductions in MAP and mean PA pressure) only after bolus injection of 50 × ED_{95}. This effect was transient in all animals (return to baseline within 5–7 min) and was not accompanied by concomitant change in peak inspiratory pressure or pulmonary compliance.

To determine whether direct myocardial effects of GW280430A contributed to the transient hypotension, indices of LV systolic and diastolic function were examined. Figure 5A depicts the hemodynamic response to 50 × ED_{95} in one animal. As is evident from the tracings, the reduction in systemic blood pressure was accompanied by increased peak AoQ and aortic dQ/dt (consistent with reduced afterload in conjunction with preserved or increased contractility), reduced end-systolic LV volume, and an increased maximal rate of LV filling during diastole. In this example, LV contractility as assessed by PWR_{max}/EDV^2 was 0.635 before GW280430A and 0.682 after. Figure 5B shows the effect of 50 × ED_{95} on LV preload and derived indices of systolic and diastolic function with all animals combined. These data indicate that on average, GW280430 has no direct cardiodepressive effect contributing to systemic hypotension. Figure 6 depicts the relation between GW280430A dose, plasma histamine concentration, and MAP 1 min after bolus injection. Coincident with the changes in MAP, the plasma histamine concentration did not increase markedly until 25 × ED_{95} (1.6 mg/kg) of GW280430A had been administered. Despite the increase in plasma histamine, there was no evidence of concomitant pulmonary vasoconstriction (mean PA pressure actually decreased slightly after 50 × ED_{95} GW280430A) or bronchoconstriction.

Discussion

GW280430A, which represents a class of nondepolarizing muscle relaxants termed *asymmetrical mixed-on-*

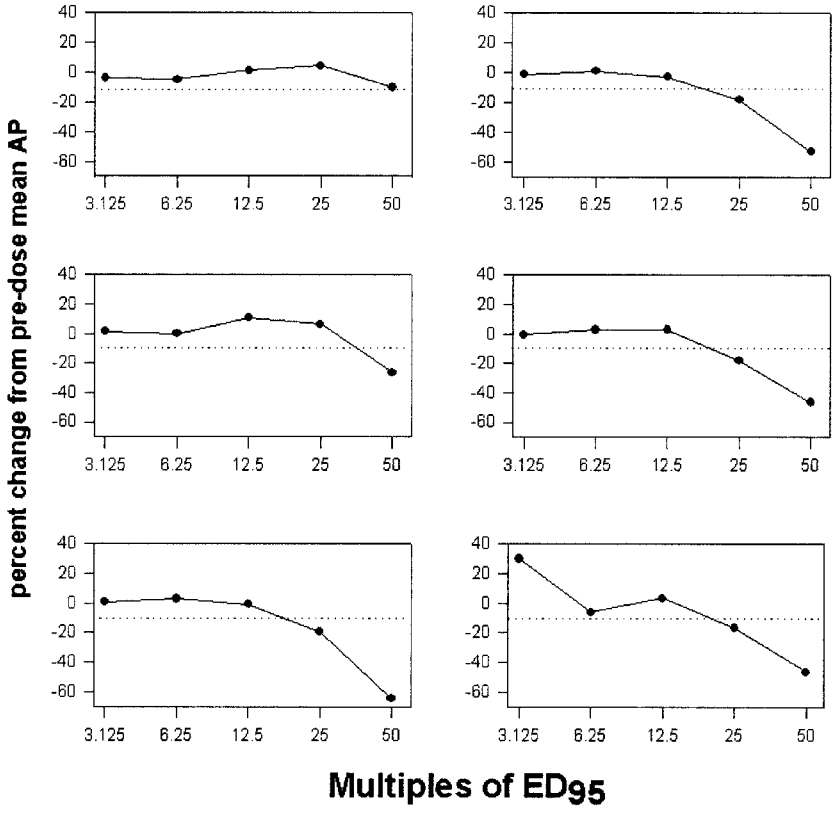

Fig. 3. Maximal change in mean arterial pressure (AP) produced by incremental bolus injection (plotted on log scale) of GW280430A in individual dogs. In four of six animals, a reduction of 10% or greater (*dotted line*) was produced by a dose representing 25 × ED_{95}.

ium chlorofumarates, has undergone preliminary evaluation in cats,[8] nonhuman primates,[4,8] and humans.[9] These studies have consistently demonstrated an ultrashort duration of action, a response attributed to two nonenzymatic processes.[4] The current study confirms a brief duration of action in beagles as well, regardless of whether the drug is administered as a bolus or *via* continuous infusion.

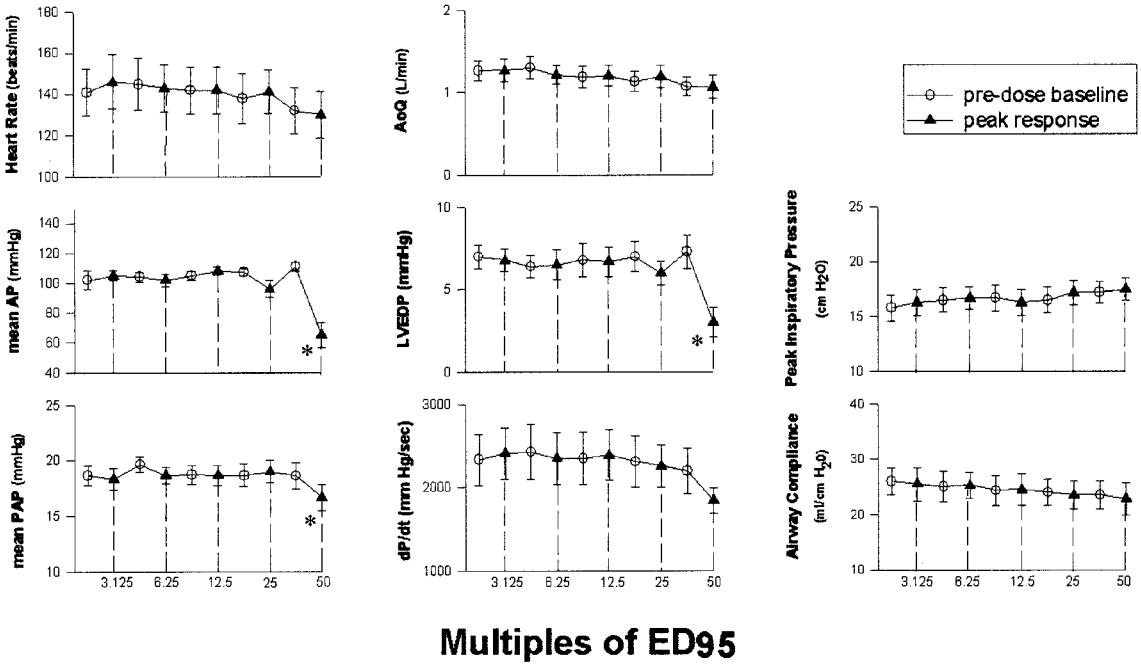

Fig. 4. Mean values for cardiopulmonary variables before and 1 min after incremental bolus injection (plotted on log scale) of GW280430A (peak response). *$P < 0.05$ in comparison with predose baseline. AoQ = mean aortic blood flow; AP = arterial pressure; dP/dt = first derivative of left ventricular pressure; LVEDP = left ventricular end-diastolic pressure; PAP = pulmonary arterial pressure.

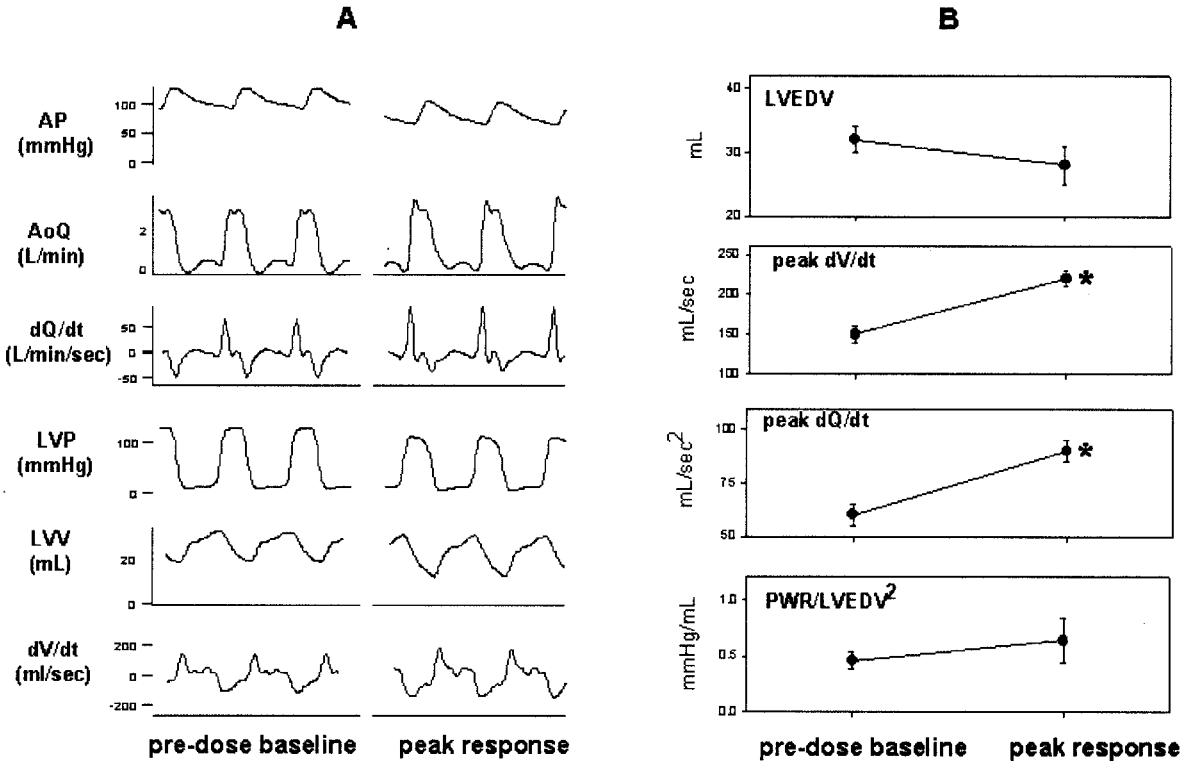

Fig. 5. (*A*) Representative hemodynamic tracings before and 1 min after (peak response) bolus injection of 50 × ED_{95} in a dog. (*B*) Mean values from six animals. * $P < 0.05$ in comparison with predose baseline. AoQ = aortic blood flow; AP = arterial pressure; dQ/dt = first derivative of AoQ (maximal acceleration); dV/dt = first derivative of left ventricular volume; LVEDV = left ventricular end-diastolic volume; LVP = left ventricular pressure; LVV = left ventricular volume; PWR = power.

The primary focus of this investigation was to expand our knowledge of potentially dose- limiting cardiopulmonary side effects associated with GW280430A. Consistent with previous studies,[10] the drug was administered in an escalating dose paradigm, with bolus injections continued until an adverse response—defined as a change of 10% or greater—was produced in one or more observed variables. Within this construct, 25 × ED_{95} produced an adverse response (a reduction in MAP) in four of six animals that was associated with an increase in plasma histamine. However, this decrease in MAP was modest (< 20%), and when dose-related changes in the average MAP from all animals were analyzed by repeated-measures analysis of variance, the decline produced by 25 × ED_{95} was not of statistical significance.

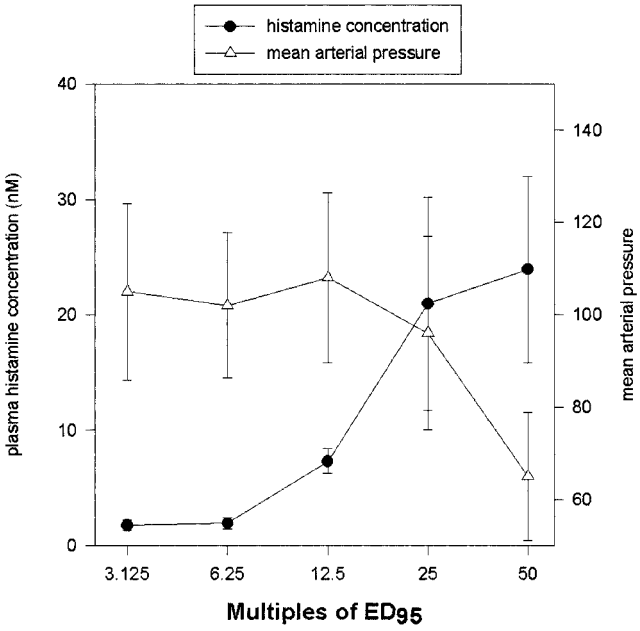

Fig. 6. Dose-related changes (plotted on log scale) in mean arterial pressure and plasma histamine.

Multiple other aspects of the cardiopulmonary response to bolus doses of GW280430A are also noteworthy. First is the observation that even after large doses, there was no evidence of either bronchoconstriction or pulmonary vasoconstriction. Second is the fact that GW280430A did not produce significant change in ascending aortic blood flow. Thus, despite even the substantial transient decrease in MAP produced by 50 × ED_{95}, cardiac output was maintained, indicating marked vasodilation as a major factor in systemic hypotension. Consistent with this response, evaluation of LV inotropy and lusitropy, along with maximal acceleration of blood in the aorta during GW280430A-induced hypotension, failed to show direct depressive effects on myocardial performance.

The results of the study need to be interpreted in the context of certain limitations. First is the relatively narrow scope of this investigation. Although the data clearly characterize multiple aspects of the cardiopulmonary response to GW280430A, they were obtained from a small sample size of purebred dogs anesthetized with thiopental, isoflurane, and nitrous oxide. Accordingly, caution must be exercised in extrapolating our findings to more genetically diverse dog populations, different species, or subjects anesthetized with a different technique. Species considerations in particular are highlighted by the fact that the pharmacokinetics of any drug can vary widely, thus influencing possible side effects as well as inducing species-dependent variation in the onset and the duration of action. For example, although mivacurium produces a relatively brief neuromuscular blockade in most species, the drug exhibits a long duration of action in beagles,[11] a finding that complicates direct comparison of novel short-acting drugs with mivacurium in this animal model. A second limitation relates to the escalating dose paradigm used to elicit potential cardiopulmonary responses. Although the data show that in some animals, large doses of GW280430A produce a transient decrease in MAP coincident with histamine release, the data also show that smaller doses can elicit detectable release of histamine without hemodynamic change. Accordingly, when administered in escalating doses, GW280430A may produce depletion of histamine stores and decrease the magnitude of release that would have occurred if a large dose, such as 25 $\times$ ED_{95}, were administered first.

In summary, the data show no hemodynamic effect of GW280430A until a dose at least 25 times greater than the ED_{95} is administered as a rapid intravenous bolus. This effect is transient, seems to be the result of histamine release with secondary systemic vasodilation, and is not accompanied by changes in peak inspiratory pressure or pulmonary compliance.

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