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*DPI – 3290 (II): A MIXED OPIOID AGONIST WITH POTENT ANTINOCICEPTIVE ACTIVITY AND LIMITED EFFECTS ON RESPIRATORY FUNCTION

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*DPI-3290 is currently being co-developed with Organon Corporation as Org 41793

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Running Title: Pharmacology of DPI-3290

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Abbreviations: TIPP, (Tyr-Tic-Phe-Phe); nor-BNI, (nor-binaltorphimine); CTOP, (cyclic [D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2]); DPDPE, (cyclic [D-Pen^2,D-Pen^5]enkephalin); MPE, maximal percent effect; NLX, naloxone; NTI, naltrindole; DPI-3290, (+) 3-((α-R)-α-((2S,5R)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-N-(3-fluorophenyl)-N-methylbenzamide; U69593, (5α,7α,8α)-(-)-N-methyl-N-(7-(1-pyrrolidinyl)-1-oxaspiro(4.5) dec-8-yl)burzeneacetamide; DAMGO, D-Ala, MePhe, Gly(ol) enkephalin.

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Abstract

Allyl-2,5-dimethyl-1-piperazines have been of interest as analgesic agents for the management of moderate-to-severe pain. In this study we compared the antinociceptive properties and respiratory depressant activity of one such agent, DPI-3290, with those of established narcotic analgesics, morphine and fentanyl. Intravenous administration of DPI-3290 in conscious laboratory rats increased antinociception in a dose-dependent manner with a corresponding ED_{50} value of 0.05 $\pm$ 0.0072 mg/kg. Simultaneous measurement of arterial blood gas in animals treated with DPI-3290 demonstrated dose-dependent increases in pCO_2 with an ED_{50} value of 0.91 $\pm$ 0.22 mg/kg. In comparison, morphine and fentanyl increased antinociception in rats with ED_{50} values of 2.01 $\pm$ 0.0005 and 0.0034 $\pm$ 0.00024 mg/kg, respectively, and the ED_{50} value for morphine-induced changes in pCO_2 was 4.23 $\pm$ 0.72mg/kg whereas, the ED_{50} value for fentanyl-induced changes in pCO_2 was 0.0127 $\pm$ 0.0035mg/kg. A separate series of experiments were designed to examine the effects of DPI-3290 on *mu*-opioid receptor induced antinociception and hypercapnia. Intravenous bolus doses of DPI-3290 that ranged from 0.2-1.0 mg/kg had no effect on antinociception mediated by alfentanil (2µg/kg/min, iv) but reduced hypercapnia by approximately 50%. Results from these studies demonstrate the equivalent antinociceptive efficacy of DPI-3290, morphine and fentanyl but dramatic differences in the hypercapnia that antinociceptive doses of these drugs produce. When measured simultaneously, DPI-3290 had a 18.2-

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fold difference in the ratio comparing the ED_{50} value for antinociception with the ED_{50} value for changes in pCO_2; this ratio was 2.1 for morphine and 3.7 for fentanyl. Furthermore, DPI-3290 reduced the alfentanil-mediated hypercapnia without any effect on antinociception. Taken together, the balanced opioid agonist activity of DPI-3290 may provide a means of powerful analgesia while mitigating the *mu*-opioid receptor mediated hypercapnia.

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Pain management is a major therapeutic challenge for which opioid analgesics are the mainstay in the treatment of moderate-to-severe pain (Mason, 1999; Inturrisi, 1990; Clots and Nahata, 1991; Holder *et al*., 1995). These agents, which have powerful analgesic action, have been used for 200 years in spite of their narrow therapeutic index and their participation in deleterious drug-drug interactions. Adverse effects commonly associated with the use of narcotic analgesics include, respiratory depression, nausea and vomiting, constipation, bradycardia, hypotension, hallucinations, euphoria, tolerance, dependence and addiction potential (Chang, 1984; Inturrisi, 1990; Reisine and Pasternak, 1993). The most life threatening of these adverse effects is respiratory depression, which accounts for a majority of the resulting deaths, linked to the use of narcotic analgesics (Inturrisi, 1990; Reisine and Pasternak, 1993).

Despite numerous efforts to develop analgesics with improved safety profiles, morphine and fentanyl remain the most widely used narcotic analgesics. Both morphine and fentanyl elicit their pharmacologic action by selectively binding to and activating the *mu*-subtype of the opioid receptor (McGilliard and Takemori, 1978; Chang, 1984). Recently, the analgesic activity of several non-peptide *delta*-opioid receptor agonists has been described (Chang et al., 1993) and there is experimental evidence to support the potential clinical advantages of a compound with combined *mu* and *delta* opioid receptor agonist activities (O'Neill et al., 1997). In studies with laboratory rats, additive analgesic actions were measured when fentanyl was administered in combination with the *delta*-opioid receptor agonist, BW373U86. Not only was there additive analgesic activity in these studies, but co-administration was also shown to diminish fentanyl-induced muscle

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rigidity (straub tail) and attenuate the BW373U86-mediated seizures. Other investigators have demonstrated that prolonged co-administration of morphine and BW373U86 attenuated the development and expression of morphine-induced dependence and tolerance in rats (Lee et al., 1993). Perhaps most importantly, it has been reported that alfentanil-mediated antinociception was not altered by co-administration with BW373U86 and in these studies the alfentanil-mediated respiratory depression was significantly attenuated (Su et al., 1998). Taken together, these data may suggest that a compound with mixed *mu*- and *delta*-opioid receptor agonist activity may have utility in achieving a similar, or even a greater degree of analgesia and fewer adverse effects when compared with fentanyl or morphine.

DPI-3290 {chemical name, (+) 3-((α-R)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl-N-(3-fluorophenyl)-N-methylbenzamide} was designed and synthesized to act at multiple opioid receptors. We have previously described the nanomolar binding affinity and comparable potency of DPI-3290 to inhibit contractions in electrically stimulated smooth muscle islolated from laboratory animals (Gengo et al., submitted). These actions are consistent with the compounds mixed opioid agoinst properties. We herein describe the *in vivo* pharmacologic actions of the mixed *delta*- and *mu*-opioid receptor agonist DPI-3290 and its subsequent antinociceptive and respiratory effects in laboratory rats.

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Methods

Arterial blood gas and antinociceptive studies. Male albino Wistar Hannover rats (HDS Madison, WI) weighing 200-300g were anesthetized with 2% isoflurane in a 30% O_2 and 70% N_2O vehicle. Under aseptic conditions the femoral artery and external jugular vein were isolated and a cannula consisting of Silastic tubing was introduced into the lumen of vessel. The cannulas were anchored with a silk suture and the incisions were closed with proline. Anesthetic gases were removed and the rats were allowed to recover in a plastic restrainer for 60 min prior to administration of test compound and simultaneous measurement of arterial blood gasses and antinociceptive responses.

Following intravenous administration of test article, arterial blood was drawn into a syringe with heparin from the femoral artery catheter. The volume of blood taken was 0.15 ml. The syringe was capped and the blood was analyzed immediately (Ph/Gas Analyzer Synthesis 25 Model, Instrumentation Laboratory). Caution was taken to avoid exposing the arterial blood sample to atmospheric air. The blood exposed to atmospheric air at the tip of the syringe was expelled prior to blood gas determinations.

The antinociceptive assay used in these studies was the standard tail-pinch test described previously (Wong et al., 1992; Le Bars et al., 2001). Briefly, the test was performed with the rat in a plexiglass restrainer and pressure from an artery clamp was placed on the tail (one inch from the tip). The clamp remained in place until an escape response occurred (i.e. tail-flick

or vocalization) or a maximum time of 20 seconds had elapsed. The escape response latency was recorded by means of a stopwatch.

Data were converted from the latency response time or unit into a maximal-percent-effect score (MPE) as described below.

$$MPE = \frac{\text{response time} - \text{basal time}}{\text{maximum time} - \text{basal time}} \times 100$$

Alfentanil infusion studies. Male albino Wistar Hannover rats (HDS Madison, WI) weighing 200-300g were anesthetized with 2% isoflurane in a 30% O_2 and 70% N_2O vehicle. A cannula consisting of Silastic tubing was introduced into the lumen of the femoral artery and 2 others introduced into the lumen of each external jugular vein. The cannulas were anchored to the vessel with a silk suture and the incisions were closed with proline. Anesthetic gases were removed and rats were allowed to recover in a plastic restrainer for 60 min prior to continuous intravenous infusion (2µg/kg) with alfentanil. Alfentanil-mediated changes in pCO_2 and MPE were allowed to stabilize prior to co-administration of intravenous DPI-3290. Simultaneous measurement of arterial blood gasses and antinociceptive responses were measured during the 60 min alfentanil infusion and then for the 30 minutes that followed.

Sources of drugs. Alfenta **(**alfentanil HCl) was purchased from Taylor Pharmaceuticals, Decatur, IL. Morphine, fentanyl, and all other chemicals were reagent grade and purchased from Sigma Chemical Co., St. Louis, MO. DPI-3290 was synthesized at Ardent Research Laboratories and

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Burroughs Wellcome Co., Research Triangle Park, NC using standard protocols**.**

Calculations and Statistics. Pharmacologic data were analyzed by linear regression of the linear portion of the dose-response curves to determine the EC_{50} value using the computer program, Prism (GraphPad Software Inc., 5755 Oberlin Drive, # 110, San Diego, CA 92121, USA). The ED_{50} values and SEM were calculated and represent the curve fit.

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Results

To compare and differentiate the actions of DPI-3290 with morphine and fentanyl, studies were designed to simultaneously measure each compounds antinociceptive properties and respiratory depressant activity in conscious laboratory rats. When DPI-3290 was administered intravenously to conscious rats the most striking effect was a dose-related increase in antinociception. The ED_{50} value for DPI-3290-mediated antinociception was 0.05 $\pm$ 0.0072mg/kg. In addition, this compound also produced an increase in pCO_2, but changes in pCO_2 resulted at markedly higher doses in relationship to those that elicited antinociception, especially when compared to morphine or fentanyl. For example, in conscious rats, the ED_{50} value for hypercapnia mediated by DPI-3290 was 0.91 $\pm$ 0.0072, a dose that was 18.2-fold higher than its ED_{50} value for antinociception. In comparison, the ED_{50} value for morphine and fentanyl-mediated antinociception in conscious rats was 2.01 $\pm$ 0.0005 and 0.0034 $\pm$ 0.0002, respectively; doses that were only 2.1-fold lower than the ED_{50} value for hypercapnia with morphine and 3.7-fold lower for the ED_{50} value for hypercapnia with fentanyl (table 1).

Gengo *et al*. (2002) have demonstrated that DPI-3290 binds selectively and with high affinity to *delta*-, *mu*-, and *kappa*-opioid receptors. These investigators also reported greater potency at *mu*-opioid receptors when DPI-3290 was compared to morphine and similar activity when compared to fentanyl. As shown in figures 1, 2, and 3, the efficacy of DPI-

3290-mediated effects on tail pinch latency (MPE) was similar when compared to morphine or fentanyl in that full antinociceptive action was measured in rats treated with DPI-3290, morphine, or fentanyl. Also illustrated in figures 1, 2, and 3 are the concomitant changes in pCO_2, pO_2, and pH that were mediated by DPI-3290, morphine and fentanyl. As would be expected, the opioid-mediated antinociceptive actions of these compounds were linked to changes in pCO_2, pO_2, and pH, but with differing potencies when standardized to their antinociceptive activity. For example, at the maximum doses that were tolerated by rats in these studies with DPI-3290, morphine and fentanyl, the percent increase in pCO_2 elicited by DPI-3290 was 78.6 $\pm$ 5.9 % whereas the increase elicited by morphine was 72.8 $\pm$ 8.8 % and that by fentanyl was 112.3 $\pm$ 13.9 %. However, even as the changes in pCO_2 mediated by maximal tolerated doses of DPI-3290, morphine, and fentanyl appear similar, they are markely different when compared with their antinociceptive activity. The 78.6 $\pm$ 5.9 % increase in pCO_2 mediated by DPI-3290 was measured at a dose that was 40-times its ED_{50} value for antinociception. Similar changes in pCO_2 were elicited by maximal tolerated doses of morphine but at a dose that was only 4-times its ED_{50} value for antinociception and by fentanyl which was 12-times its ED_{50} value for antinociception.

Because the selective *delta* opioid peptides (DPDPE and deltorphin II) and non-peptide (BW373U86) agonists inhibited the hypercapnia induced by the continuous infusion of the selective *mu*-opioid analgesic alfentanil (Su et al., 1998), it has been proposed that *delta*-opioid receptor agoinsts mitigate the hypercapnia resulting from narcotic analgesics. We investigated this possibility by measuring the effects of DPI-3290 on alfentanil-mediated hypercapnia and antinociception in rats. A 2 μg/min intravenous infusion of

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alfentanil maintained full antinociception (MPE) and increased pCO_2 concentrations to 55 $\pm$ 8 mm/Hg in rats. These changes in tail pinch latency (MPE) and pCO_2 induced by alfentanil reached steady state in 20-25 minutes and were maintained throughout the 60-minute duration of this study (fig. 4). Bolus intravenous doses of DPI-3290 that ranged from 0.2 mg/kg to 1.0 mg/kg resulted in no change in tail pinch latency (MPE) when co-administered during the alfentanil infusion. Bolus intravenous doses of DPI-3290 that ranged from 0.2 mg/kg to 1.0 mg/kg reversed the alfentanil-induced elevation in pCO_2 by approximately 50% at all doses tested. Thirty minutes after the alfentanil infusion had been terminated, tail pinch latency (MPE) and arterial blood gas (pCO_2) both returned to baseline values.

The increases in antinociception (MPE) and arterial blood gas (pCO_2) induced by intravenous doses of DPI-3290, morphine, and fentanyl are illustrated in figure 5. As demonstrated in this figure, all three compounds are strong antinociceptive agents with varying potency: fentanyl > DPI-3290 >> morphine. The most striking distinction between DPI-3290 and these compounds is the difference in the dose-response relationships that compare antinociception with hypercapnia.

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Discussion

DPI-3290 is a novel mixed opioid receptor agonist with potent antinociceptive activity (Gengo et al., submitted). Unlike morphine or fentanyl that demonstrate high affinity binding and intrinsic activity at *mu*-opioid receptors (Wolozin and Pasternak, 1981, Alt et al., 2002), DPI-3290 has high affinity binding and intrinsic activity at *delta*-, *mu*- and *kappa*-opioid receptors providing a new approach for managing the analgesic needs of patients with moderate-to-severe pain. The high affinity binding and intrinsic activities of DPI-3290, morphine and fentanyl at *mu*-opioid receptors provide an adequate explanation for their antinociceptive pharmacologies (Law and Loh, 1999). That is, by modulating the presynaptic release of neurotransmitters such as acetylcholine, norepinephrine, serotonin, dopamine or substance P they alter synaptic transmission (Satoh and Minami, 1995; Childers, 1991). These changes in neurotransmitter release have been linked to the actions of receptor operated potassium channels, adenylate cyclase activity, and intracellular free ionized calcium concentrations (Schiller et al., 1992; Fan and Crain 1995; Fan et al., 1993; Jin et al., 1994). Together, these changes in neuronal signal transduction that are mediated by *mu*-opioid receptor agonists hyperpolarize the membrane potential, blunt voltage sensitive calcium channel activity and dampen the cellular excitability of neurons thus inhibiting painful stimuli.

Although the literature is replete with reports that describe how *delta*-opioid receptors agonists decrease the hypercapnic effects of *mu*-opioid agonists without changing their antinocicpetive actions (Negri et al., 1995; Su et al., 1998; O'Neill et al., 1997; Rossi et al., 1994; He and Lee, 1998), the molecular mechanism that underlies these effects is not fully elucidated.

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This would suggest that a compound with balanced agonist activity at *delta*- and *mu*-opioid receptors could have a pharmacologic profile distinct from currently available narcotic analgesics. DPI-3290 is an agonist with high affinity binding characteristics at opioid receptors that does not distinguish between the *delta*-, *mu*- or *kappa* subtypes. This is in contrast to morphine or fentanyl, which are relatively selective agonists with high affinity binding at the *mu*-subtype of the opioid receptor. If the mixed opioid receptor agoinst activity of DPI-3290 mitigates the hypercapnia associated *mu*-opioid receptor agonists, it might be anticipated that DPI-3290 would have strong antinociceptive action with reduced hypercapnia when compared to morphine or fentanyl. Indeed, the 18.2-fold difference between the ED_{50} value for changes in pCO_2 and the ED_{50} value for antinociception elicited by DPI-3290 supports this hypothesis. These results with DPI-3290 can be contrasted with the 2.1-fold difference for morphine and the 3.7-fold difference with fentanyl, further supporting the concept that mixed opioid agonists are strong antinociceptive agents with less hypercapnia when compared to selective *mu*-opioid receptor agonists like morphine or fentanyl.

It is notable that high concentrations of DPI-3290, morphine and fentanyl increase pCO_2 in laboratory animals. This is a characteristic of all opioid narcotic analgesics and has been purported to result from *mu*-opioid receptor activation. Inasmuch as the magnitude of changes in pCO_2 elicited by maximal tolerated doses of DPI-3290, morphine and fentanyl were quantitatively similar, their relationship to the ED_{50} value for antinociception was not. These findings further support the notion that mixed opioid receptor agonists like DPI-3290 are potent antinociceptive agents that appear to act as competitive antagonists of the *mu*-opioid receptor mediated changes in pCO_2.

In the clinical setting the most life threatening adverse effect associated with the use of narcotic analgesics is respiratory depression. Today, all clinically available narcotic analgesics used to treat moderate-to-severe pain produce respiratory depression at therapeutic doses. Reports demonstrating CTOP blockade of antinociception and hypercapnia resulting from continuous alfentanil infusion argues that *mu*-opioid receptors underlie these effects. This would explain why therapeutic doses of narcotic analgesics are linked to respiratory depression. Reports that *delta* opioid receptor agonists have no direct effect on respiration further support the notion that respiratory depression following morphine or fentanyl treatment is mediated by *mu* opioid receptors. Although DPI-3290 is a potent *mu*-opioid receptor agonist, the difference in its effects on respiratory depression at comparable analgesic doses of morphine or fentanyl are likely to be valid. Not only were the effects of DPI-3290 on respiratory depression distinct from those measured with morphine or fentanyl, but also DPI-3290 reversed the respiratory depression induced by continuous intravenous alfentanil infusion. Because DPI-3290 improved rather than added to the respiratory depression of alfentanil, this mixed opioid receptor agonist mitigates not only the respiratory depression coupled to its strong *mu*-opioid agonist activity but also the respiratory effects of narcotic analgesics like alfentanil.

The characteristic that defines the paramount difference between DPI-3290 and narcotic analgesics like morphine or fentanyl is the marked difference in the doses that elicit its antinociceptive and respiratory depressant activities. Because the most life threatening adverse effect associated with the use of narcotic analgesics for moderate-to-severe pain is respiratory depression, a drug with an appropriate separation between analgesia and hypercapnic activities could relieve severe pain with a broader

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therapeutic index. In this regard, the mixed opioid receptor agonist activity of DPI-3290 and its antinociceptive and hypercapnic pharmacologies is evidence that indicates the likelihood for achieving such a goal. Studies are underway to assess the efficacy and therapeutic index of DPI-3290 in more diverse models of pain and narcotic induced adverse effects and to determine its potential as an analgesic for the treatment of patients with moderate-to-severe pain.

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TABLE 1
ED_{50} values for opioid effects on antinociception and hypercapnia in laboratory rats.

The results summarized represent the mean ± S.E.M. from 6-8 independent animals.

Compound	Tail-Pinch (mg/kg)	PCO_2 (mg/kg)	Safety Ratio PCO_2 : Tail-Pince
DPI-3290	0.05±0.0072	0.91±0.22	18.2*
Morphine	2.01±0.0005	4.23±0.72	2.1^{+}
Fentanyl	0.0034±0.0002	0.0127±0.0035	3.7

*$P < 0.05$ vs morphine or fentanyl, $^{+}P > 0.05$ vs fentanyl.

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Figure 1. Time course for the changes in MPE, pCO_2, pO_2, and pH induced by intravenous administration of DPI-3290 at the doses indicated. At the time points outlined in the figure, tail-pinch testing and arterial blood samples were collected and analyzed as described in the "Methods and Materials".

Figure 2. Time course for the changes in MPE, pCO_2, pO_2, and pH induced by intravenous administration of morphine at the doses indicated. At the time points outlined in the figure, tail-pinch testing and arterial blood samples were collected and analyzed as described in the "Methods and Materials".

Figure 3. Time course for the changes in MPE, pCO_2, pO_2, and pH induced by intravenous administration of fentanyl at the doses indicated. At the time points outlined in the figure, tail-pinch testing and arterial blood samples were collected and analyzed as described in the "Methods and Materials".

Figure 4. Time course for effects of the mixed opioid agonist DPI-3290 on MPE and pCO_2 responses in alfentanil-infused rats. Alfentanil was intravenously infused at 2μg/kg/min. At the time points outlined in the figure, tail-pinch testing and arterial blood samples were collected and analyzed as described in the "Methods and Materials'.

Figure 5. The dose-response relationship for antinociception (open symbols) and changes in pCO_2 (closed symbols) for DPI-3290 (circles), morphine (squares), and fentanyl (triangles) in laboratory rats. Values represented are the mean $\pm$ S.E.M. of six separate experiments.

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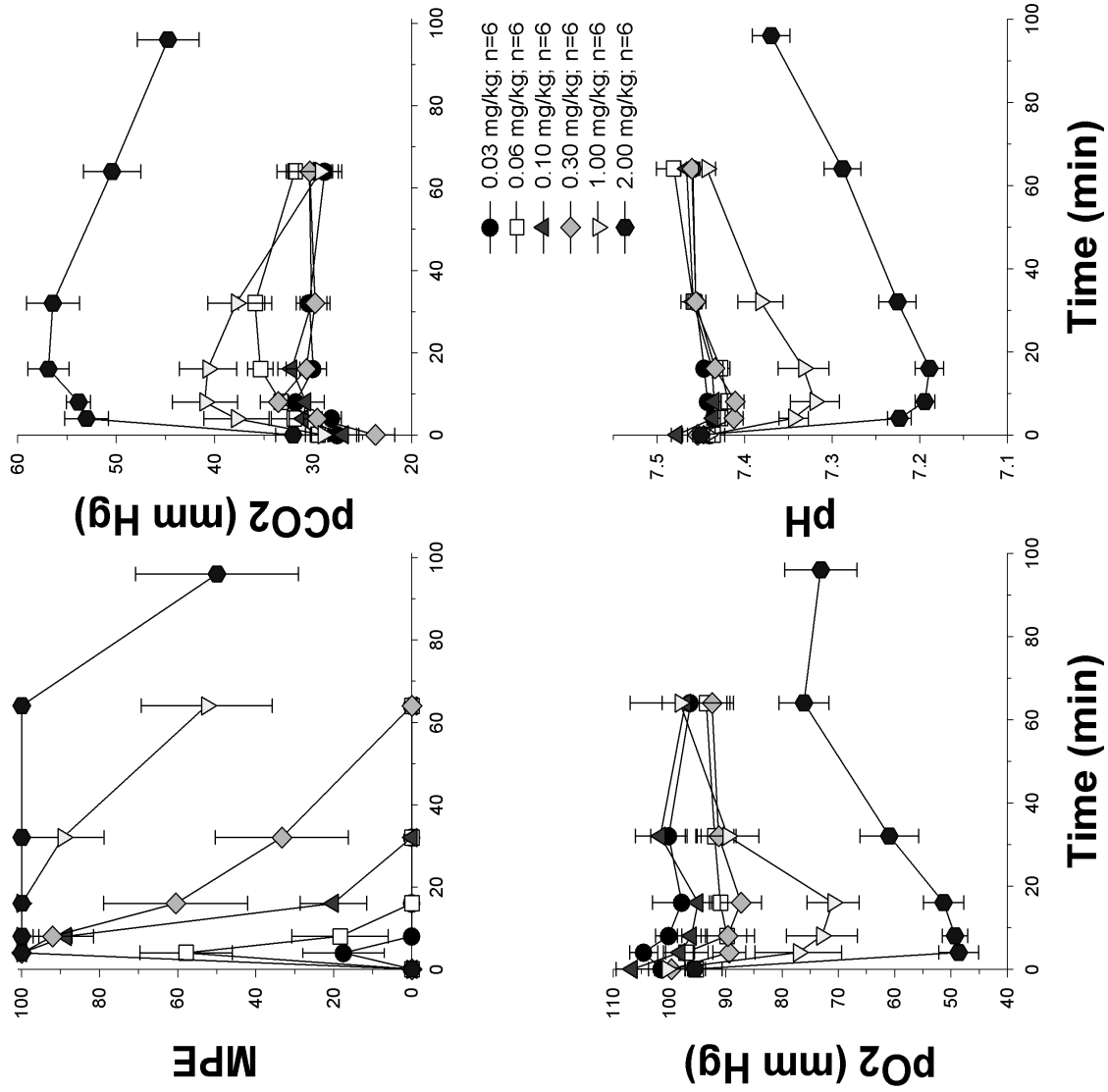

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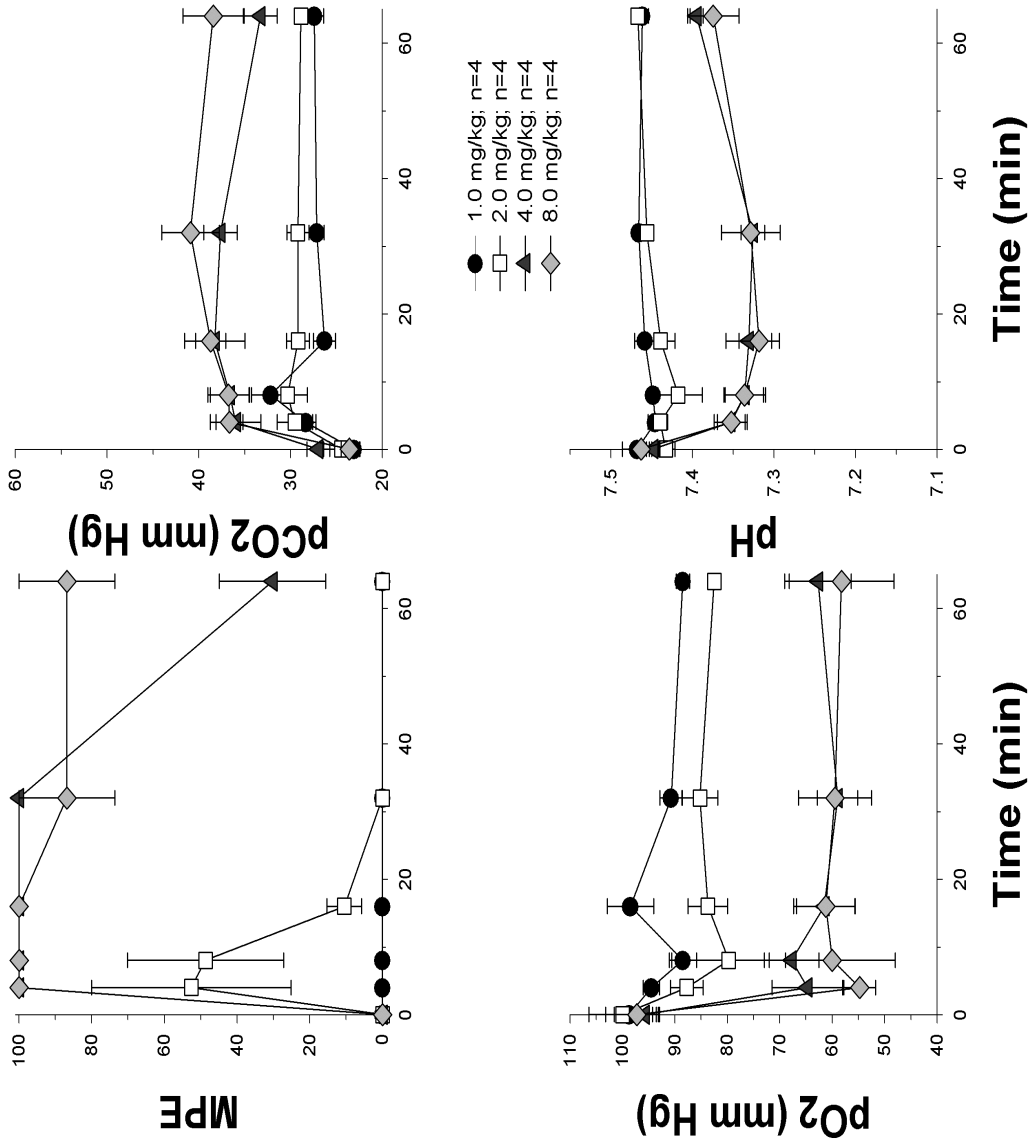

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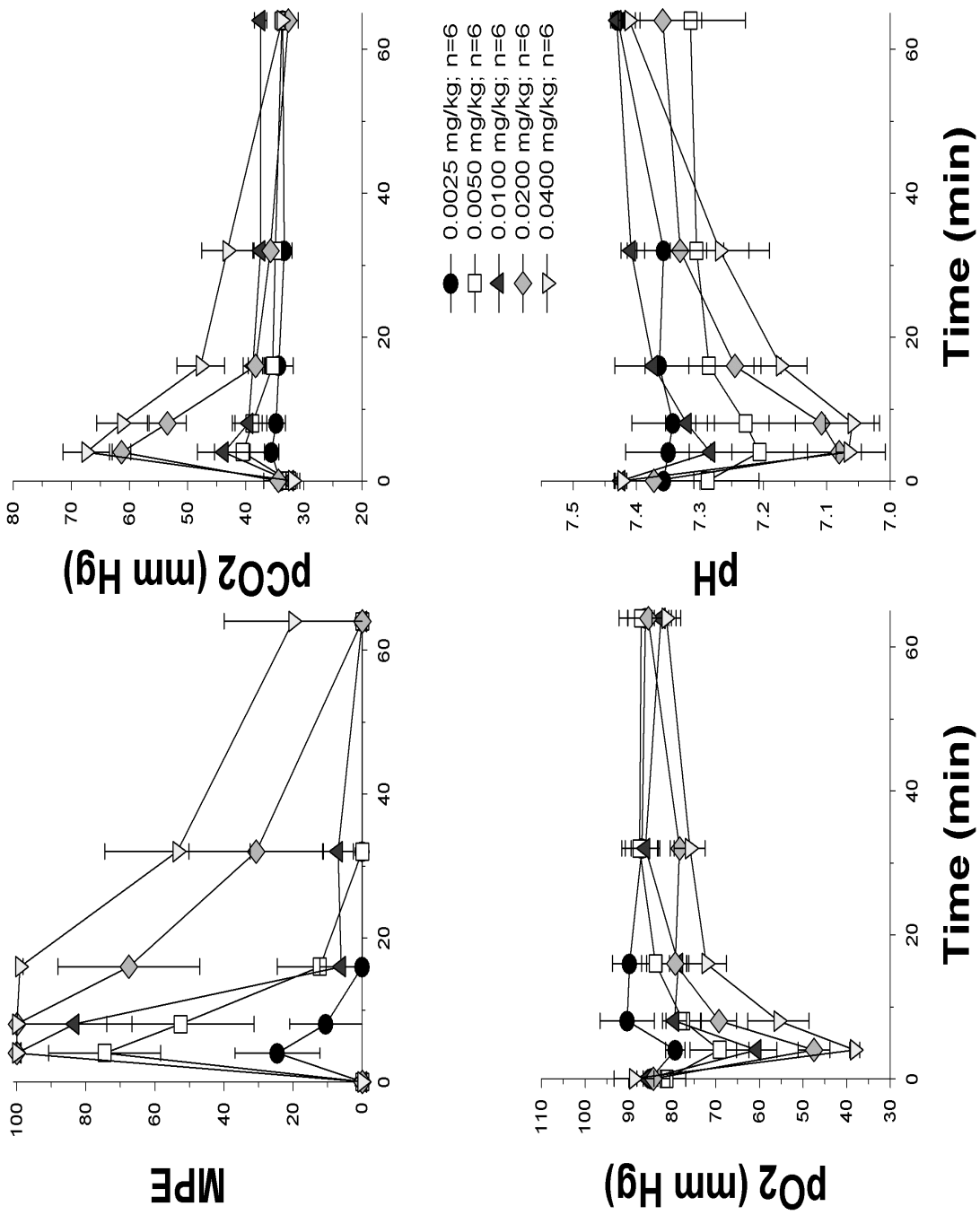

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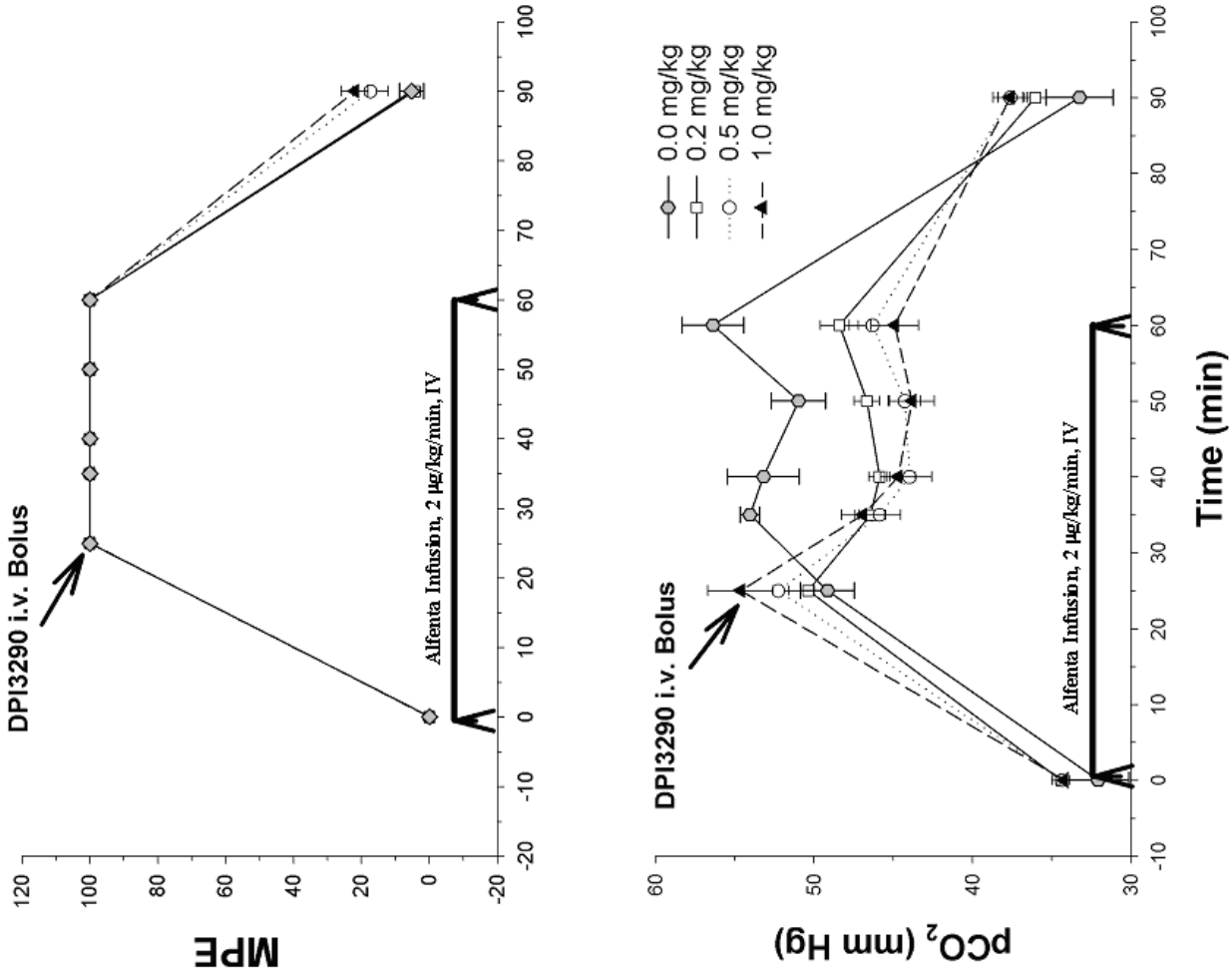

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