Potencies of Doxapram and Hypoxia in Stimulating Carotid-body Chemoreceptors and Ventilation in Anesthetized Cats

Robert A. Mitchell, M.D.,* and Dorothy A. Herbert, A.B.†

The effects of doxapram on carotid chemoreceptor activity and on ventilation (phrenic-nerve activity) were tested before and after denervation of the peripheral chemoreceptors in cats. Doxapram was found to be a potent stimulus to the carotid chemoreceptors; the stimulation produced by 1.0 mg/kg doxapram, iv, equaled that produced by a PaO₂ of 38 torr. Doxapram also increased phrenic-nerve activity in doses as low as 0.2 mg/kg, iv. After denervation of the peripheral chemoreceptors, doxapram in doses as large as 6 mg/kg failed to stimulate ventilation. It is concluded that (in anesthetized cats) doxapram in doses of less than 6 mg/kg increases ventilation by direct stimulation of the carotid, and, probably, the aortic, chemoreceptors, not by a direct effect on the medullary respiratory center. (Key words: Receptors, chemoreceptors; Hypoxia, doxapram; Analectics, doxapram; Ventilation, doxapram.)

Many physicians reject use of "respiratory stimulants" to treat respiratory failure resulting from chronic pulmonary disease or drugs.1,2 Rejection is based on the lack of specificity of central nervous system stimulants such as nikethamide, ethamivan, pentylentetrazol, Prethcamide, dimefline, amphetamine, and picrotin.3 The doses of these analeptics required to increase ventilation significantly are close to those that cause convulsions.

One drug may not be subject to the above reservations. Doxapram (1-ethyl-4 (2-
morpholinoethyl)-3, 3-diphenyl-2 pyrrolidinone hydrochloride hydrate), introduced about 12 years ago,4 is reported to reverse postoperative hypoxia and hypercapnia and has been advocated for therapy of respiratory failure resulting from chronic pulmonary disease, where oxygen therapy may be expected to exaggerate hypercapnia and acidosis.5,6 In addition to its effect on respiration, doxapram causes an increase in arterial blood pressure and arousal after anesthesia. Unlike other analeptic agents, doxapram has a large margin of safety; the therapeutic ratio (convulsive dose/ventilatory stimulating dose) is about 20–40, compared with 2–4 for other analeptic agents, suggesting that doxapram has a direct, selective stimulatory effect on respiratory neurons at doses that do not stimulate nonrespiratory units. This is supported by the work of Funderburk et al.,7,8 who demonstrated that doxapram increased neural activity in medullary respiratory centers in intact cats without stimulating higher centers. Kato and Buckley9 also reported direct respiratory center stimulation, but in addition found significant stimulation of the peripheral chemoreceptors. Recently, Hirsh and Wang10 demonstrated that doxapram stimulated medullary inspiratory units more than nonrespiratory units. After carotid chemoreceptor denervation, this specificity was lost, and higher doses were necessary to stimulate ventilation. They concluded that therapeutic doses of doxapram increased ventilation through stimulation of carotid chemoreceptors. To evaluate this proposal, we compared the effects of doxapram on ventilation before and after denervating carotid and aortic chemoreceptors with those of hypoxia. We also recorded afferent chemosensory activity from a strand of carotid sinus nerve and compared the effect of doxapram on...
chemosensory impulse activity with that of hypoxia.

**Methods**

We anesthetized ten cats, weighing 3.5–6.2 kg, by intraperitoneal injection of chloralose (40 mg/kg) and urethane (200 mg/kg). We paralyzed the cats with gallamine triethiodide (20 mg initially, repeated hourly) and mechanically ventilated them. The general procedure for the studies was as follows. We initially determined the respiratory responses to graded hypoxia and to intravenously administered doses of doxapram (0.2–6.0 mg/kg), using the integrated effluent phrenic-nerve activity as our index of ventilation.12 We then cut the aortic depressor and carotid sinus nerves. After denervation, and four hours after the last dose of doxapram, we again tested the integrated phrenic-nerve responses to hypoxia and doxapram. In addition, we determined the carotid chemoreceptor afferent nerve responses to graded hypoxia and intravenous injection of doxapram (0.2–10 mg/kg).

The specific experimental details were as follows. We performed a tracheostomy and cannulated one femoral artery and vein. In three cats we also position a cannula in the right common carotid artery, by way of the lingual artery, just below the carotid sinus. The larynx and esophagus were transected in the neck and reflected rostrally. We exposed the right and left carotid sinus and aortic depressor nerves, as well as the right phrenic nerve, which was transected low in the neck. We covered the entire area of the neck dissection with paraffin warmed to body temperature. The proximal end of the cut phrenic nerve was placed on bipolar platinum electrodes.

Following the initial studies, we cut the aortic depressor and carotid sinus nerves. We placed the distal end of the right carotid sinus nerve on a dissecting platform, separated the nerve into fine strands, and recorded action potentials from them with platinum electrodes. The amplified signals from the phrenic nerve and a strand of the carotid sinus nerve that contained only active chemoreceptor afferent fibers were displayed on an oscilloscope and photographed. The amplified signals also were fed to a pulse height selector and ratemeter, and the phrenic-nerve signal also was fed to the "leaky" integrator. The output of the ratemeters and integrator, the arterial blood pressure, and tracheal Pco2, (measured by infrared analyzer) were recorded on a Grass polygraph. In addition, the ratemeter output
was continuously measured by a frequency counter and a digital recorder. Rectal temperature was monitored constantly and maintained near normal by an infrared lamp. The ventilatory and carotid body chemosensory responses to various levels of $P_{O_2}$ at a constant $P_{CO_2}$ were determined by measuring the discharge of the nerve fiber with each different mixture of inspired gas until the frequency remained constant for two successive minutes. During the following minute we recorded the nerve activity on film and withdrew a sample of femoral arterial blood, which we analyzed for $pH$, $P_{CO_2}$, and $P_{O_2}$. Arterial blood $pH$ and $P_{CO_2}$ were maintained near constant by intravenous infusion of NaHCO$_3$ and by altering the level of mechanical ventilation, respectively. The average $pH$ was 7.33 ± .09 and the average $P_{CO_2}$ was 37 ± 7.0 torr. Only those data points in which $P_{CO_2}$ varied less than 6 torr and $pH$ varied less than 0.1 unit were accepted for construction of the $P_{O_2}$ response curves for a given cat.

**Results**

**Comparison of Carotid Chemoreceptor Stimulation by Hypoxia and by Doxapram**

Rapid intravenous injection of 0.2 to 10.0 mg/kg doxapram increased carotid chemoreceptor activity within 8 ± 2 seconds (mean ± SD) (figs. 1 and 2). Peak activity occurred 4–10 seconds after onset of increased activity, and gradually declined to control level in 2 to 20 or more minutes, depending on the dose (table 1). Between the first and second minutes after intravenous injection in cats breathing air (average $P_{O_2}$ 84 torr), the chemoreceptor afferent nerve activities produced by 0.2, 1.0, 2.0, 3.0, 6.0, and 10.0 mg/kg doxapram equalled those produced by $P_{aO_2}$'s of 42, 38, 36, 35, 33, and 31 torr, respectively (table 1, figs. 3 and 4). The stimulation produced by doxapram was not significantly reduced by hypoxia ($P_{aO_2}$ 400 torr), but was reduced by severe hypoxia.

**Fig. 2.** Effect of doxapram, 1.0 mg/kg, intravenously, with the carotid sinus and aortic nerve intact (A) and after section of nerves (B). In both A and B the top trace is blood pressure, the second trace is a record of the integrated phrenic nerve activity (IP) and in B the third trace is a ratemeter record of activity from a strand of carotid sinus nerve. Units for IP are arbitrary. Note the delay in the increase in blood pressure after section of the carotid sinus nerve.
TABLE 1. Duration and Magnitude of Stimulation by Doxapram

<table>
<thead>
<tr>
<th>Dosapram mg/kg iv</th>
<th>Equivalent PaO₂ (torr)</th>
<th>Duration (Min)</th>
<th>Equivalent PaO₂ (torr)</th>
<th>Duration (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>42 ± 9</td>
<td>1.5 ± 0.4</td>
<td>48 ± 7</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>1.0</td>
<td>38 ± 3</td>
<td>4.7 ± 1.9</td>
<td>41 ± 4</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>2.0</td>
<td>36 ± 3</td>
<td>5.9 ± 2.2</td>
<td>39 ± 3</td>
<td>5.1 ± 1.4</td>
</tr>
<tr>
<td>3.0</td>
<td>35 ± 2</td>
<td>10.3 ± 3.4</td>
<td>37 ± 3</td>
<td>9.4 ± 2.1</td>
</tr>
<tr>
<td>6.0</td>
<td>33 ± 4</td>
<td>18.0 ± 5</td>
<td>35 ± 2</td>
<td>15.8 ± 4.9</td>
</tr>
<tr>
<td>10.0</td>
<td>31</td>
<td>&gt;20</td>
<td>—</td>
<td>—</td>
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</tbody>
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* Means ± SD.

(PaO₂ < 50 torr) (fig. 5). Intracarotid injection of doxapram in doses subthreshold for stimulation by intravenous injection (0.05 mg/kg) produced significant carotid chemoreceptor stimulation lasting 2–3 minutes (fig. 6). Injection of as much as ten times the threshold dose for intravenous stimulation into the descending aorta failed to stimulate the carotid chemoreceptors.

COMPARISON OF VENTILATORY RESPONSES TO HYPOXIA AND DOXAPRAM

The latency, magnitude, and duration of increased integrated phrenic-nerve activity (IPA) following doxapram administration were similar to the chemoreceptor response (fig. 2). One to two minutes after intravenous injection of 0.2, 1.0, 2.0, 3.0, and 6.0 mg/kg doxapram, IPA's were equal to those produced by PaO₂'s of 48, 41, 39, 37, and 35 torr, respectively (figs. 7 and 8). IPA increased primarily through increased activity per breath (increased V̇E); frequency of breathing increased 10 per cent or less.

Following carotid and aortic chemoreceptor denervation, hypoxia had no significant effect on IPA until PaO₂ was below 30 torr, where apnea occurred. Intravascular injection of doxapram in doses smaller than 6 mg/kg resulted in no significant stimulation of IPA, and in two cats, markedly decreased IPA for 30–45 seconds after injection. Doses larger than 6 mg/kg increased IPA slightly by increasing frequency of breathing (fig. 8).

CARDIOVASCULAR RESPONSES

In the intact cat, intravenous injection of doxapram caused an increase in arterial blood pressure without a significant change in heart rate. The onset occurred 8 ± 2 (mean ± SD) seconds after injection, reached its peak 7 to 12 seconds later, and gradually returned to normal in 2–40 minutes, depending on the dose (fig. 2). One mg/kg. iv, increased arterial pressure from 156/98 (SD ± 12/7) to 205/145 (SD ± 26/18) torr at the peak of the response. After denervation of the peripheral chemoreceptors, the onset of the blood pressure response occurred 16–20 seconds following injection, and peak response was delayed to 20–35 seconds after the onset of a pressure rise. Intravenous injection of 1.0 mg/kg increased arterial pressure from 175/115 (SD ± 30/19) to 210/145 (SD ± 34/26) torr without altering heart rate.

Discussion

Doxapram has been said to increase ventilation by a direct and selective action on the respiratory centers, to increase blood pressure (primarily by stimulation of brainstem vasomotor areas), and to hasten arousal postoperatively. The statement regarding ventilation comes from the work of Funderburk and Alphin,49 who reported that doxapram in doses as low as 0.2 mg/kg increased the electrical activity in the medullary inspiratory and expiratory centers, while ten times this dose was needed to produce minimal effects in cortical and spinal areas. Kato and Buckley10 recorded blood pressure, respiratory rate, and heart rate in dogs and concluded that the respiratory and pressor effects of doxapram were mediated both by central stimulation and by stimulation of the aortic and carotid chemoreceptors. Hirsh
and Wang\textsuperscript{11} likewise found that doxapram given intravenously in doses of 0.05 to 5.0 mg/kg increased the firing of medullary respiratory units and minute ventilation. Nonrespiratory units were stimulated at doses larger than 0.5 mg/kg. However, the increase in firing rate of respiratory units was greater than that of nonrespiratory units. After carotid chemoreceptor function was eliminated, this specificity of action on respiratory units was lost. Doses of doxapram smaller than 1.0 mg/kg were now ineffective in stimulating respiratory neurons. Our studies, where comparable, are in agreement and confirm the results of Hirsh and Wang.\textsuperscript{11} We found that 0.2, 1.0, and 6.0 mg/kg doxapram in the intact cat increased phrenic-nerve activity to 158, 186, and 207 per cent of control. This is similar to the 150, 170, and 250 per cent increases in minute ventilation in response to 0.25, 1.0, and 5.0 mg/kg doxapram reported by Hirsh and Wang.\textsuperscript{11} After carotid body denervation, they obtained significant increases in ventilation and neural

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**FIG. 3.** Effect of arterial oxygen tension (P\textsubscript{aO\textsubscript{2}}) on impulse activity of a strand of carotid sinus nerve, represented by solid circles, and the effects of various doses of doxapram (dose in mg/kg in brackets), represented by open circles, all at 84 torr P\textsubscript{aO\textsubscript{2}}. Average of data from four cats.

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**FIG. 4.** Effects of various doses of doxapram on impulse activity of a strand of carotid sinus nerve, recorded with the cats breathing room air. Figures in brackets indicate the oxygen tension (in torr) causing equivalent activity without doxapram. Average data from four cats.
activity (both respiratory and nonrespiratory neurons) with doses of 1.0 mg/kg or more. However, at these doses the increases in ventilation and respiratory neural activity were markedly reduced from those in the intact cat and the specificity of action upon respiratory units was eliminated. In our studies we did not observe statistically significant stimulation of phrenic-nerve activity until doses larger than 6 mg/kg were given. We conclude, as did Hirsh and Wang, that low doses of doxapram increase respiration by stimulation of carotid, and possibly aortic, chemoreceptors. Higher doses are necessary to stimulate respiration in the absence of peripheral chemoreceptors. The early cardiovascular response to doxapram also results from activation of peripheral chemoreceptors. However, the site of stimulation that results in the delayed response which produces a pressor response not significantly different from that produced by the chemoreceptors remains unknown.

The prolonged response to doxapram is in sharp contrast to the brief responses produced by other drugs that stimulate the carotid body, such as sodium cyanide (figs. 1 and 6). The prolonged effect of doxapram might be the result of 1) continued recirculation of the drug; 2) specific binding by tissues, including chemoreceptor sites; or 3) nonspecific binding by tissues, including chemoreceptor sites. The third possibility seems most likely, since intravenously

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**FIG. 6.** Ratemeter records of activity from a strand of the carotid sinus nerve following (A) intracarotid arterial injection of 10 micrograms sodium cyanide, (B) intracarotid arterial injection of 0.025 mg/kg doxapram, and (C) the same dose of doxapram given intravenously.
Fig. 7. Effects of arterial oxygen tension (P_{O_2}) on respiration as represented by integrated phrenic-nerve activity before (●) and after (▲) section of the aortic and carotid sinus, compared with the effects of various doses of doxapram (figures indicating mg/kg dose in brackets) before (○) and after (▲) denervation, all superimposed at 84 torr P_{O_2}.

Fig. 8. Effects of various doses of doxapram on integrated phrenic-nerve activity (index of respiration) before (●) and after (▲) denervation of the carotid sinus nerves. The figures in brackets indicate the arterial oxygen tensions (in torr) that cause equivalent activity without doxapram. All doxapram points were recorded with the cat breathing room air.

administered doses of doxapram that fail to stimulate the carotid chemoreceptors are effective in producing prolonged responses when injected into the carotid artery. Thus, recirculation of the drug is not the cause of its prolonged response. Also, ten times the intravenous dose necessary to produce a significant response is needed to produce a response when injected into the descending aorta, indicating that the drug is rapidly removed from the circulation.

Our failure to observe any change in respiratory rate (as measured by the frequency of phrenic-nerve activity bursts) or heart rate might be due to the rapid (two–three times normal) rate at which we mechanically ventilated the paralyzed cats with low tidal volumes. This effectively disrupts those vagal afferents from the lung that are normally synchronous with respiration.

We did not evaluate the arousal reportedly produced by this drug. Part or all of the arousal effect could be mediated by the peripheral chemoreceptors, since near-maximal stimulation of the carotid
chemoreceptors in awake dogs can produce
convulsions.12

Doxapram has been tested in man as an
adjunct to the treatment of chronic obstruc-
tive pulmonary disease and postoperative
respiratory depression. Moser et al.6 and
Edwards and Leszczynski7 found the drug to
be effective in a number of patients. The
latter found it to be the most potent of the
so-called respiratory stimulating drugs they
tested. Winnie et al.1-3 found that the drug
stimulated respiration in seven of ten post-
operative patients. However, Virtue14 found
the drug ineffective in reversing postoperative
hypoxia in patients studied in Denver.

It is not proper to suggest the clinical use
of this drug in man on the basis of studies in
cats; however, certain of our observations
may be pertinent to the use of this drug and
to future studies in man. In cats, doses
corresponding to the recommended
therapeutic dose in man (0.5–2 mg/kg) pro-
duce a response equalling 85–95 per cent of
the maximal response. Funderburk et al.9
report that doses in the therapeutic range do
not produce subcortical or spinal stimulation;
therefore, the large therapeutic ratio of this
drug probably results from the selective
carotid body stimulation. In the absence of
peripheral chemoreceptor activity, the speci-
cicity of action on respiration is lost, and
therefore the respiratory stimulating effect
and the therapeutic ratio both are dimin-
ished.

The dependence of the respiratory stimula-
tion produced by doxapram upon the carotid
bodies suggests the possible use of doxapram
in the measurement of carotid chemoreceptor
function. If it can be demonstrated that the
ventilatory response to doxapram correlates
with the respiratory response to hypoxia in
normal subjects, in persons living at high
altitude, and in patients after removal of the
carotid bodies, the drug may provide a quick,
simple measure of carotid body function. It
would have advantages over currently used
drugs such as sodium cyanide or Veratridine
in that 1) its long duration of action provides
time to obtain a relatively stable measure-
ment of ventilation, and 2) its effect does not
impose hazards such as those of cyanide or
hypoxia itself.

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