INTERACTIONS BETWEEN MORPHINE AND DOXAPRAM IN THE RABBIT AND MOUSE

S. M. GREGORETTI AND B. J. PLEUVRY

SUMMARY

Certain actions of doxapram, administered alone and in combination with morphine, have been examined in the rabbit and the mouse. Single doses of doxapram were capable of stimulating respiration in both species. There was an increase in tidal volume in the rabbit and an increase in respiratory rate in the mouse. In both species the duration of action of single doses of doxapram was less than 15 min. In morphine-treated rabbits and mice single doses of doxapram affected neither the time course nor the intensity of the respiratory depression. In the rabbit repeated doses of doxapram did not produce tachyphylaxis with respect to the effect on tidal and minute volumes, and effectively reversed the respiratory depressant actions of morphine. The usefulness of this action must be balanced against the enhanced toxicity of doxapram observed in morphine-treated mice.

The advisability of using analeptic agents has been questioned seriously (Goodman and Gilman, 1975) because of the low therapeutic index of these compounds. However, interest in analeptic agents has increased following the introduction of doxapram, a pyrrolodinone derivative. It is claimed that this compound is a relatively specific respiratory stimulant and has a wider margin of safety than other analeptic compounds (Rockwell and Greene, 1963). Luscombe and Nicholls (1971) demonstrated, in a variety of species, that the ratio of the convulsant dose to the respiratory stimulant dose was higher for doxapram than for any other analeptic agent used in their study.

The potential usefulness of doxapram is enhanced by the observation that, unlike nikethamide, it does not antagonize the analgesic actions of the narcotic analgesics (Gupta and Dundee, 1974). Furthermore, Gawley and others (1976) found that the administration of doxapram to patients receiving morphine reduced significantly the incidence of postoperative cough, the expectoration of purulent sputum and the degree of hypoxaemia. Such improvements with doxapram occurred whether the drug was given as a single dose or as an infusion. The efficacy of the single dose is surprising in view of the rapid metabolism of doxapram (Bruce et al., 1965) and its reported evanescent action (Goodman and Gilman, 1975), although Ramamurthy, Steen and Winnie (1975) observed that, in man, single doses of doxapram could antagonize the respiratory depressant actions of pethidine for several hours. Thus doxapram may behave differently when acting in combination with narcotic analgesics than when given alone.

The present study was designed to investigate the interactions between doxapram and morphine in the rabbit and mouse, with reference to the respiratory effects.

METHODS

Respiratory measurements in rabbits

Dutch rabbits (1.5–2.5 kg) of either sex, in groups of five, were studied.

Respiratory rate and tidal volume were measured using a pneumotachograph flow transducer (5 mm, Mercury Instruments) which acted as a resistance to inspiratory flow. Measurements were made whilst applying a padded mask over the snout of the rabbit. The mask could be made airtight by the use of gentle pressure and, after a brief introductory period, rabbits tolerated the mask without significant changes in respiratory frequency.

Pressure changes across the flow transducer were measured using a pneumotachograph flow transducer (5 mm, Mercury Instruments) which acted as a resistance to inspiratory flow. Measurements were made whilst applying a padded mask over the snout of the rabbit. The mask could be made airtight by the use of gentle pressure and, after a brief introductory period, rabbits tolerated the mask without significant changes in respiratory frequency.

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After stable control values had been obtained either the drug under test or saline was injected into the lateral ear vein. Readings were taken continuously for the first 5 min at least, and then at 15-min intervals. In general the results were expressed as percentage change from control values, but some absolute values are presented below to allow comparison with other studies.

$P_{CO_2}$ and pH were measured, before and at 15-min intervals (initially 5-min in the case of doxapram) after administration of the drug, from arterialized venous blood, collected anaerobically in heparinized capillary tubes from the lateral ear vein of the rabbit. The ear was shaved, warmed and massaged gently before sampling to ensure brisk circulation. A Radiometer blood micro system (BMS3 Mk 2) was used. Actual bicarbonate values were obtained using an acid-base slide rule.

In all experiments the rabbits were restrained gently, by wrapping loosely in a laboratory coat, and the ears were kept warm throughout the experiment by placing an electric light close to them. In order to disturb the animals as little as possible during the experiment, rectal temperatures were not measured routinely. However, preliminary experiments, using rabbits trained to tolerate a rectal probe over a 90-min period, demonstrated no significant changes in the temperature of morphine-treated rabbits when wrapped as described above.

**Studies using mice**

The respiratory rate in mice was measured using the method described by Bousfield and Rees (1969). The snout of the mouse was placed in the barrel of a 5-ml syringe attached to a pressure transducer (Bell & Howell), which was connected to a recorder (Devices). The number of breaths in a 10-s period was counted and multiplied by 6 to give breaths per minute (b.p.m.).

**Estimations of $LD_{50}$**

$LD_{50}$ with confidence limits was calculated by the method of Litchfield and Wilcoxon (1949). Ten mice were used for each dose of drug and observations were continued over the subsequent 24 h.

Except for the $LD_{50}$ estimations, results were expressed as mean ± SEM and significance was calculated using Student's $t$ test.

**Drugs used**

The drugs used in both species were morphine hydrochloride and doxapram hydrochloride. Both drugs were dissolved in saline and were injected i.v. in the rabbit, and via the intraperitoneal route in mice. All doses refer to the salts.

In the mouse the dose of morphine ranged from 10 mg. kg$^{-1}$ to a lethal dose. The dose of doxapram ranged from 25 mg. kg$^{-1}$ to a lethal dose.

In the rabbit, however, morphine was given as a single dose of 4 mg. kg$^{-1}$ and doxapram was administered either as a single dose of 5 mg. kg$^{-1}$ or as repeated injections. These consisted of a priming dose of 5 mg. kg$^{-1}$ followed, at 5-min intervals for 60 min, by further doses of doxapram 5 mg per rabbit. This procedure was adopted to simulate an initial dose given to antagonize respiratory depression followed by an infusion of 1 mg.min$^{-1}$ to maintain the antagonism. It was not practicable to set up an infusion in an unrestrained rabbit.

In morphine-treated rabbits single doses of doxapram were injected 1 min after morphine and the repeated injections of doxapram were commenced 15 min after morphine.

**RESULTS**

**Respiratory measurements in rabbits**

Table I shows the pre-injection control values in the rabbits.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean ± SEM</th>
<th>n</th>
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<tbody>
<tr>
<td>Respiratory rate (b.p.m.)</td>
<td>105 ± 6</td>
<td>35</td>
</tr>
<tr>
<td>Tidal volume (ml)</td>
<td>4.0 ± 0.2</td>
<td>35</td>
</tr>
<tr>
<td>Minute volume (ml)</td>
<td>438 ± 30</td>
<td>35</td>
</tr>
<tr>
<td>pH (units)</td>
<td>7.46 ± 0.007</td>
<td>23</td>
</tr>
<tr>
<td>$P_{CO_2}$ (kPa*)</td>
<td>4.73 ± 0.08</td>
<td>23</td>
</tr>
<tr>
<td>Actual bicarbonate (m mol/litre)</td>
<td>24.2 ± 0.5</td>
<td>23</td>
</tr>
</tbody>
</table>

*1 kPa = 7.5 mm Hg.

Morphine 4 mg. kg$^{-1}$ caused an increase in tidal volume but a decrease in respiratory frequency and minute volume (fig. 1). This was associated with a significant increase in $P_{CO_2}$ and a decrease in pH ($P<0.001$) when compared with saline-treated control rabbits (fig. 2). These effects of morphine were maximal 15 min after the injection and did not alter significantly during the subsequent 60 min. Over this time morphine caused no significant change in actual bicarbonate.

Single doses of doxapram 5 mg. kg$^{-1}$ produced an increase in tidal volume which reached a peak about 30's after injection, was maintained for a further
MORPHINE AND DOXAPRAM INTERACTIONS IN RABBIT AND MOUSE

2 min and then returned to normal values about 6 min after the injection. Doxapram caused also a decrease in respiratory rate which was greatest 2 min after the injection and which returned to pre-injection values about 10 min after the injection. However, this single dose of doxapram did not inhibit any of the effects of morphine upon the tidal volume, respiratory frequency and minute volume even when measurements...
TABLE II. Effects of doxapram 5 mg. kg⁻¹ upon the ventilation of the normal and morphine-pretreated rabbit. Results are expressed as mean values ± SEM of groups of six rabbits.

<table>
<thead>
<tr>
<th></th>
<th>% change from pre-injection value measured 2 min after injection</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Tidal volume</td>
</tr>
<tr>
<td>Saline</td>
<td>-5.2 ± 5.9</td>
</tr>
<tr>
<td>Doxapram 5 mg.kg⁻¹</td>
<td>+44.5 ± 12.0*</td>
</tr>
<tr>
<td>Morphine 4 mg.kg⁻¹</td>
<td>-1.3 ± 9.0</td>
</tr>
<tr>
<td>Morphine 4 mg.kg⁻¹+</td>
<td>+8.6 ± 7.6</td>
</tr>
<tr>
<td>doxapram 5 mg.kg⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from saline values (P < 0.05).

were made at the time of the peak activity of doxapram (as observed in control rabbits) (table II).

Figure 3 shows the effects of repeated injections of doxapram 5 mg upon the tidal volume and respiratory frequency of the rabbit, expressed as maximum changes. The initial priming dose of doxapram caused an increase in tidal volume and a decrease in respiratory frequency (as described previously). The following “first” 5-mg dose of doxapram caused an increase in tidal volume, but its effect on respiratory rate was variable, as indicated by the large SEM. The second dose of doxapram 5 mg, however, produced no significant changes in either tidal volume or respiratory rate. The third (15-min) injection produced a small increase in tidal volume and a decrease in respiratory frequency and thereafter repeated injections of doxapram produced a consistent increase in tidal volume of about 30%. However, the depressant effects of doxapram upon respiratory frequency waned with repeated injections until the frequency was not altered significantly by the last (12th) injection of doxapram 5 mg.

Readings taken just before each injection of doxapram showed no cumulation of the actions of doxapram. After the initial “priming” dose, there was a sustained increase in tidal volume, a decrease in respiratory rate, not significantly different from that of saline-treated rabbits, and no change in minute volume (fig. 1).

The overall increase in the efficiency of respiration during repeated injections of doxapram was demonstrated by the significant decrease in P<sub>CO₂</sub> and increase in pH (P < 0.05) of the arterialized venous blood from rabbits subjected to this treatment (fig. 2).

Figures 1 and 2 show also the effects of repeated doxapram injections in rabbits pretreated with morphine 4 mg. kg⁻¹. There was a rapid antagonism, not only of the depression of respiratory frequency and minute volume induced by the morphine, but also of the increase in tidal volume. This last observation was surprising in view of the finding that doxapram increased tidal volume when administered alone.

The reversal of the respiratory effects of morphine was confirmed by measurements of arterialized venous P<sub>CO₂</sub> and pH (fig. 2). The P<sub>CO₂</sub> and pH of the morphine-treated rabbits returned to near control values following repeated injections of doxapram.

The decrease in P<sub>CO₂</sub>, increase in pH and increase in minute volume produced by the doxapram
injections in morphine-treated rabbits were consider-

ably greater than the equivalent changes produced by
doxapram injections in the control rabbits.

There were no significant changes in actual
bicarbonate concentration.

Experiments in mice

In mice it was feasible to measure respiratory rate
only. The mean pre-injection respiratory rate
(± SEM) of a group of 24 mice was 174 ± 7 b.p.m.
During the course of an experiment, control values
tended to decrease as the mice became accustomed to
being handled and to their environment. However,
this normal decrease in respiratory rate was inhibited
by doxapram 25 and 50 mg.kg⁻¹, although doxapram
100 mg.kg⁻¹ was necessary to increase significantly
the respiratory rate above pre-injection control values
(table III).

<table>
<thead>
<tr>
<th>Drug</th>
<th>% change in frequency (b.p.m.)</th>
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<tbody>
<tr>
<td>Saline</td>
<td>−27.0 ± 5.8</td>
</tr>
<tr>
<td>Doxapram 25 mg.kg⁻¹</td>
<td>−6.0* ± 6.4</td>
</tr>
<tr>
<td>50 mg.kg⁻¹</td>
<td>+6.0* ± 8.4</td>
</tr>
<tr>
<td>100 mg.kg⁻¹</td>
<td>+15.8* ± 4.3</td>
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</tbody>
</table>

*Significantly different from saline values (P<0.05).

Morphine 20 mg.kg⁻¹ reduced respiratory rate to
88 ± 5.3 b.p.m. (n = 12) at the time of its peak effect
and maintained significant respiratory depression for
120 min. None of the doses of doxapram tested
(25–200 mg.kg⁻¹ i.p.), significantly reduced the
depressant action of morphine.

A number of mice died following the combination of
doxapram 100 mg.kg⁻¹ and morphine 20 mg.kg⁻¹
and this prompted a study of the LD₅₀ for doxapram
in the presence of morphine. The results are shown in
Table IV. Death after doxapram alone occurred about
20 min after injection following violent convulsions.
After morphine alone, death occurred after a few
hours and some animals convulsed, but most exhibited
laboured breathing and cyanosis. In contrast, in mice
dying after both drugs, the circumstances of death
were less dramatic. They were sedated, piloerected
and cold and the first animals died 3–4 h after the
combined drugs had been injected.

<table>
<thead>
<tr>
<th>Drug</th>
<th>LD₅₀ (mg.kg⁻¹)</th>
<th>95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxapram alone</td>
<td>267.6</td>
<td>192.9–298.2</td>
</tr>
<tr>
<td>Doxapram in the</td>
<td>144.1*</td>
<td>115.7–185.5</td>
</tr>
<tr>
<td>presence of morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg.kg⁻¹</td>
<td>81.6*</td>
<td>56.3–108.2</td>
</tr>
<tr>
<td>Doxapram in the</td>
<td></td>
<td></td>
</tr>
<tr>
<td>presence of morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mg.kg⁻¹</td>
<td>351.1</td>
<td>326.4–367.3</td>
</tr>
</tbody>
</table>

*Significantly less than the value for doxapram alone
(P<0.05).

DISCUSSION

In previous studies from this laboratory, the minute
volume of the rabbit has been measured using a more
direct method, the Krough spirometer (Bradshaw,
Biswas and Pleuvry, 1973). The change to a pneumo-
tachograph was necessitated by the lack of kymograph
smoking facilities. However, the pre-injection abso-
lute values for minute volume and respiratory rate
were similar to those obtained by more direct
measurements and the response of the rabbits to
morphine 4 mg.kg⁻¹ was similar to that obtained on a
previous occasion (Bradshaw, Biswas and Pleuvry,
1973).

Single doses of doxapram 5 mg.kg⁻¹ produced, in
rabbits, an increase in tidal volume and a decrease in
frequency. These two actions were out of phase, in
that the increase in tidal volume occurred immediately
after injection whilst the decrease in respiratory rate
was delayed, reaching a maximum 2–3 min after
injection. Thus minute volume was increased only
transiently. The duration of all these actions of
doxapram was brief as both tidal volume and fre-
quency had returned to the control values 15 min after
injection. The short duration of action of doxapram
may be a result of its rapid metabolism (Bruce et al.,
1965) or it may reflect a “bolus effect” on respiratory
centres with subsequent redistribution (Goodman and
Gilman, 1975).

In the presence of morphine, the effects of doxa-
pram were so transient that tidal volume and frequency
were altered significantly only during the first minute
after injection. Two minutes after the injection of
doxapram, morphine-treated animals were indis-
istinguishable from morphine-treated control rabbits
not given doxapram.

A similar finding was obtained in mice. Whilst
doses of doxapram 25 mg.kg⁻¹ and more had
significant effects upon respiratory rate, none of these doses was able to inhibit the depression of respiratory rate induced by morphine. In man, Ramamurthy, Steen and Winnie (1975) have reported that a single dose of doxapram 2 mg.kg$^{-1}$ prevented the occurrence of significant respiratory depression associated with pethidine as measured by the ventilatory response to carbon dioxide. However, examination of their results shows that this action of doxapram occurred only 20 min after injection and that the difference between the results obtained with pethidine alone and pethidine with doxapram was not significant at any time after injection. However, doxapram alone did increase significantly the response to carbon dioxide in these volunteers. Thus it seems that in the rabbit and mouse, and possibly in man, single doses of doxapram have less ventilatory stimulant activity in the presence of narcotic analgesics than in the absence of these agents.

However, doxapram is used frequently as an infusion or as repeated injections. Polak and Plum (1964) reported that the cat exhibited tachyphylaxis following repeated doses of doxapram. Although the response of the rabbit to repeated doses of doxapram 5 mg was not constant, there was no trend towards a smaller response of the tidal volume as the number of injections increased. The increase in frequency after the doxapram 5 mg given 5 min after the priming dose of doxapram 5 mg.kg$^{-1}$ could be a cumulative effect of doxapram, the higher total blood concentrations causing a more generalized respiratory stimulation. However, this did not occur with subsequent 5-mg increments of doxapram and after the first 15 min rabbits treated with repeated injections of doxapram exhibited stable values for all the respiratory measurements.

In morphine-treated rabbits repeated injections of doxapram reversed not only the depression of minute volume, but also the increase in tidal volume and decrease in frequency. This contrasts with the overall increase in tidal volume and slight depression of tidal volume induced by repeated injections of doxapram in control animals. Furthermore, the increase in pH and decrease in $P_{\text{CO}_2}$ in rabbits which received repeated injections of doxapram was much greater in animals receiving morphine concurrently.

The principal effect of doxapram in the rabbit was to increase tidal volume and thus the decrease in respiratory frequency after each dose may be a compensatory phenomenon related to "poststimulation respiratory depression". The observation that the decrease in frequency followed the increase in tidal volume is consistent with this suggestion. In the presence of morphine, some of these compensatory phenomena may be depressed so that doxapram can stimulate respiration more effectively. However, this could not account for the apparent lack of action of single doses of doxapram in the presence of morphine in the rabbit and mouse.

Thus, the experiments on respiration indicate that whilst, in the presence of morphine, single doses of doxapram are without effects on respiration, repeat injections of doxapram provide a complete reversal of most facets of the respiratory depressant activity of morphine.

This positive finding must be tempered by the enhanced toxicity of doxapram in morphine-treated mice. It is interesting that the death of the mice after the combination of drugs, as opposed to their administration separately, should be delayed by several hours. Observation of the animals showed no obvious potentiation of the acute effects of either drug (convulsions or respiratory depression). The doses of morphine used, 10 and 20 mg.kg$^{-1}$, were not excessive for the mouse since the lethal dose is in the range of 350 mg.kg$^{-1}$. The mechanism of this enhanced toxicity is being investigated with some urgency, but no further information is available.

It is to be hoped that this interaction is murine-specific, but in view of the current vogue for morphine-doxapram mixtures and the reports of doses of doxapram in excess of 1 g being used in man (Newell et al., 1969) it may be advisable to limit doses until the situation has been clarified.

ACKNOWLEDGEMENTS

We are grateful to A. H. Robins Company Ltd for the supply of doxapram and to Miss S. Maddison for invaluable technical assistance. S. M. G. was in receipt of a Rotary Foundation Educational Award.

REFERENCES


**WECHSELWIRKUNGEN ZWISCHEN MORPHIUM UND DOXAPRAM BEI KANINCHEN UND MÄUSEN**

ZUSAMMENFASSUNG


**INTERACTION DE LA MORPHINE ET DU DOXAPRAM SUR LE LAPIN ET LA SOURIS**

**RESUME**

Certaines actions du doxapram, administré seul et en combinaison avec de la morphine, ont été observées sur la souris et le lapin. Des doses uniques de doxapram ont été capables de stimuler la respiration sur les deux espèces. Il y a eu une augmentation du volume courant chez le lapin et une augmentation de la fréquence respiratoire chez la souris. Dans les deux espèces, la durée de l'action des doses uniques de doxapram a été inférieure à 15 min. Sur les lapins et les souris traitées à la morphine, les doses uniques de doxapram n'ont affecté ni le cours du temps ni l'intensité de la dépression respiratoire. Sur le lapin, les doses répétées de doxapram n'ont pas produit de tachyphylaxie en ce qui concerne l'effet sur le volume courant et le volume minute, mais elles ont effectivement inversé l'action respiratoire déprimante de la morphine. L'utilité de cette action doit être opposée à la forte toxicité du doxapram que l'on a observée sur les souris traitées à la morphine.

**INTERACCIONES ENTRE LA MORFINA Y DOXAPRAM EN EL CONEJO Y EN EL RATON**

**SUMARIO**

Ciertas acciones del doxapram, administrado solo y en asociación con la morfina, han sido examinadas en el conejo y en el ratón. Dosis únicas de doxapram fueron capaces de estimular la respiración en ambas especies. Se produjo un aumento en el volumen corriente en el conejo y un aumento en la frecuencia respiratoria en el ratón. En ambas especies la duración de las dosis únicas de doxapram produjo una acción que se mantuvo durante menos de 15 minutos. En conejos y ratones tratados con morfina las dosis únicas de doxapram no afectaron ni el transcurso de tiempo ni la intensidad de la depresión respiratoria. En el conejo, dosis repetidas de doxapram no produjeron tachyphylaxia con respecto al efecto sobre el volumen corriente y minuto, invirtiendo eficazmente las acciones depresoras de la ventilación causadas por la morfina. La utilidad de esta acción deberá contrapesarse frente a la toxicidad aumentada del doxapram que se observó en los ratones tratados con morfina.