RESPIRATORY EFFECTS OF A NEW OPIATE ANALGESIC, R 39209, IN THE RABBIT: COMPARISON WITH FENTANYL

J. H. BROWN, B. J. PLEUVRY AND B. KAY

SUMMARY
The respiratory effects of R 39209, a new short-acting analgesic, were studied and compared with those of fentanyl, in the rabbit. Minute volume, respiratory frequency and pH, Pco₂, and standard bicarbonate of arterialized venous blood were measured. R 39209 had an earlier peak effect and shorter duration of action than fentanyl, but otherwise the respiratory effects of the two drugs were similar. Fentanyl was between 2 and 3.5 times more potent than R 39209. Repeated doses of R 39209 produced reproducible peak effects even when only 10 min was allowed between administrations.

R 39209, a new, potent, short-acting analgesic agent is currently undergoing both clinical and pharmacological investigation (De Castro et al., 1979). Its chemical structure is similar to that of fentanyl (fig. 1).

![Chemical structure of R 39209 and fentanyl](image)

Early reports (Niemegeers, 1977) indicated that R 39209 was approximately four times less potent than fentanyl and a comparative clinical investigation (De Castro, 1977) showed that, in man, R 39209 has a more rapid onset of action and an earlier peak effect than fentanyl, but its duration of action was three times shorter. The safety index for equipotent doses is similar to that of fentanyl.

All reported administrations of R 39209 in man have been accompanied by neuromuscular blockade (suxamethonium for short and pancuronium for longer procedures) and within this concept of "analgesic anaesthesia" (De Castro, 1977), it seemed that R 39209 had no obvious advantages over fentanyl except for short procedures (5–10 min) when the duration of respiratory depression in the period after operation was shorter.

This study compared R 39209 with fentanyl, in respect to effects on respiration in the spontaneously breathing conscious rabbit. In addition selected doses of the two drugs were given repeatedly, over short time intervals, and the reproducibility of the response determined.

METHODS
Twenty-eight Dutch rabbits weighing between 1.5 and 3.2 kg were studied. Sixteen were used in physiological measurements of respiration and 12 in the studies of blood-gas tensions.

Tidal volume, respiratory frequency and minute volume were measured in the conscious rabbit using a face-mask, one-way valve system and pneumotachograph (Khanna and Pleuvry, 1978). After each experimental session the apparatus was calibrated for tidal volume using a small animal ventilator set at appropriate respiratory frequencies.

Rabbits were placed in an adjustable restraining box with the head projecting through a circular orifice. A 25-gauge Butterfly needle was inserted into a lateral ear vein. After stable recordings were obtained the rabbit was given saline, a selected dose of fentanyl or R 39209. As far as possible each rabbit was allocated a single dose of fentanyl, R 39209 or saline on a random basis so that no rabbit received the same dose of drug twice. Rabbits were not used more frequently than at 2-week intervals. The injection was given over a


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period of 60–75 s to minimize the risk of convulsion. Continuous recordings were taken for at least 15 min after injection.

Pco$_2$ and pH measurements were determined (Radiometer micro system (BMS3 Mk 2)) using arterialized venous blood from a lateral ear vein collected in heparinized capillary tubes (Khanna and Pleuvry, 1978). Standard bicarbonate was calculated using a Siggaard-Andersen nomogram assuming a normal haemoglobin concentration. The ears were kept warm throughout the sampling period by proximity to an electric light. Under control conditions, Po$_2$ of blood obtained by this method was 13.21 ± 0.13 kPa (n = 57).

Constant temperature and humidity were maintained in the operating theatre throughout each investigation. All injections, recordings and blood sampling were performed by the same person (J.H.B.) and the blood-gas analyser was operated by the same assistant.

The results obtained from the pneumotachograph trace are expressed as a percentage change from pre-injection values, whereas blood-gas results are expressed as absolute change. Significance was assessed using Student's $t$ test.

Repeated injections were given at regular intervals corresponding to the time-course of the drug: 10 min for R 39209 and 15 min for fentanyl. Four separate doses were given, and continuous recordings taken for 40 min and 60 min respectively. Control rabbits were given saline repeatedly.

**Drugs and doses**

The drugs used were R 39209 2, 4, 6, 8 and 10 μg kg$^{-1}$, and fentanyl 1.25, 2.5 and 5 μg kg$^{-1}$. Studies using repeated injections were confined to R 39209 6 μg kg$^{-1}$ given at 10-min intervals and fentanyl 2.5 μg kg$^{-1}$ given at 15-min intervals.

**RESULTS**

**Single injections**

Both R 39209 and fentanyl produced dose-dependent changes in respiratory frequency and minute volume. The maximum depression of respiratory frequency after the injection of R 39209 occurred earlier than that of fentanyl ($P < 0.01$: Mann-Whitney $U$ test). The peak effect in most rabbits given R 39209 was at 3 min, whilst that for fentanyl was at 5 min. Accordingly, the means for each dose at these respective times were used to calculate the dose–response curves (fig. 2). R 39209 2 μg kg$^{-1}$ had no significant effect, but 4 μg kg$^{-1}$ produced significant decreases in respiratory frequency for up to 5 min. There were significant decreases in both respiratory frequency and minute volume for up to 5 min

![Graph](image.png)
following 6, 8 and 10 µg kg\(^{-1}\). Greater doses of R 39209 invariably produced convulsions and even 8 and 10 µg kg\(^{-1}\) produced fatal convulsions if injected rapidly.

Fentanyl 1.25 µg kg\(^{-1}\) caused some significant decreases in respiratory frequency and minute volume for up to 10 min, as did 2.5 and 5 µg kg\(^{-1}\) for up to 15 min. The time course of the effects of R 39209 10 µg kg\(^{-1}\) and fentanyl 5 µg kg\(^{-1}\) on minute volume are illustrated in figure 3 and the longer duration of action of fentanyl demonstrated.

The changes in P\(\text{CO}_2\) after R 39209 2, 6 and 10 µg kg\(^{-1}\) (table I) were significant up to 5 min after injection, but had returned to control values by 10 min. Fentanyl also produced significant increases in P\(\text{CO}_2\), which took 20 min to return to control values following both doses. The concur-

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**Fig. 3.** Time-courses of the effects of fentanyl 5 µg kg\(^{-1}\) (○) and R 39209 10 µg kg\(^{-1}\) (■) on the minute volume of the rabbit. Results are expressed as mean percentage change (± SEM) from pre-drug values (n = 4). *Significantly different from saline control values (P < 0.05).

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**Table 1.** Changes in blood P\(\text{CO}_2\) (kPa) after single injections of fentanyl and R 39209 (mean ± SEM). *Significantly different from saline (P < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Fentanyl (µg kg(^{-1}))</th>
<th>R 39209 (µg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-injection</td>
<td>3.89 ± 0.08</td>
<td>4.01 ± 0.11</td>
</tr>
<tr>
<td>Post-injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min</td>
<td>+0.96 ± 0.20*</td>
<td>+0.61 ± 0.17*</td>
</tr>
<tr>
<td>3 min</td>
<td>+0.78 ± 0.08*</td>
<td>+0.53 ± 0.20*</td>
</tr>
<tr>
<td>5 min</td>
<td>+0.67 ± 0.13*</td>
<td>+0.60 ± 0.14*</td>
</tr>
<tr>
<td>10 min</td>
<td>+0.54 ± 0.13*</td>
<td>+0.64 ± 0.10*</td>
</tr>
<tr>
<td>15 min</td>
<td>+0.54 ± 0.14*</td>
<td>+0.78 ± 0.09*</td>
</tr>
<tr>
<td>20 min</td>
<td>+0.49 ± 0.14*</td>
<td>+0.68 ± 0.25</td>
</tr>
</tbody>
</table>
Repeat injections

Fentanyl produced a stepwise decrease in respiratory frequency throughout the 60-min period (fig. 4). However, there was a progressive increase in tidal volume such that the changes in minute volume were not significantly different from those of the saline controls.

R 39209 showed more distinct and regular changes in respiratory frequency (fig. 5). In contrast to fentanyl, tidal volume decreased slightly over the 40-min period, and the time-course of the changes in minute volume resembled closely that of respiratory frequency.

Saline controls showed a gradual decrease in respiratory frequency over 60 min (e.g. decrease of 16.7% (SEM ± 3.92) at 60 min).

Maximum changes in $P_{CO_2}$ after repeat injections of both R 39209 and fentanyl are shown in table II; the effect was reproducible. However, by the third and fourth doses the $P_{CO_2}$ had not returned to control values before the next injection (table III).

Fig. 4. Effect of repeated injections of fentanyl 2.5 $\mu$g kg$^{-1}$ on the respiratory frequency of the rabbit. Fentanyl was injected at 15-min intervals. Results are expressed as mean percentage change (±SEM) from pre-drug values ($n = 4$). *Significantly different from control animals given repeated injections of saline ($P < 0.05$).

Fig. 5. Effect of repeated injections of R 39209 6 $\mu$g kg$^{-1}$ on the respiratory frequency of the rabbit. R 39209 was injected at 10-min intervals. Results are expressed as mean percentage change (±SEM) from pre-drug values ($n = 7$). *Significantly different from control animals given repeated injections of saline ($P < 0.05$).
TABLE II. Maximum change in blood PCO₂ (kPa) after repeated injections of fentanyl or R 39209. Results are mean change from control (±SEM) (n = 5). *Values significantly different from saline (P < 0.05)

<table>
<thead>
<tr>
<th>Before drug</th>
<th>Fentanyl 2.5 μg kg⁻¹</th>
<th>R 39209 6 μg kg⁻¹</th>
<th>Saline 4.03 ±0.16</th>
</tr>
</thead>
<tbody>
<tr>
<td>After drug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose 1</td>
<td>+0.82 ±0.11</td>
<td>+0.74 ±0.10</td>
<td>+0.39 ±0.30</td>
</tr>
<tr>
<td>Dose 2</td>
<td>+0.84 ±0.10*</td>
<td>+0.63 ±0.06*</td>
<td>0.00 ±0.24</td>
</tr>
<tr>
<td>Dose 3</td>
<td>+0.93 ±0.33*</td>
<td>+0.55 ±0.06*</td>
<td>-0.10 ±0.18</td>
</tr>
<tr>
<td>Dose 4</td>
<td>+0.63 ±0.12*</td>
<td>+0.59 ±0.13*</td>
<td>-0.01 ±0.19</td>
</tr>
</tbody>
</table>

R 39209 showed a distinct pattern of change in pH which did return to pre-injection values after each 10-min period.

Neither saline nor fentanyl produced significant changes from their pre-injection values in standard bicarbonate (paired t-test). However, after the second dose of R 39209, the rabbits exhibited an increase in standard bicarbonate which was significant (P < 0.05) for most of the experimental period. For example, 40 min after the beginning of the experiment, rabbits treated with R 39209 had increases in standard bicarbonate of +1.42 (SEM ±0.36) mmol litre⁻¹ compared with saline control values of -0.36 (SEM ± 0.54) mmol litre⁻¹.

DISCUSSION
The potency ratio between fentanyl and R 39209 varied from 2 : 1 with the higher doses to 3.5 : 1 with the lower doses (fig. 2). Since previous studies (Janssen preclinical research report) of the analgesic effects of R 39209 in rats and dogs had shown fentanyl to have four times the potency of R 39209, it may be that the potency ratio for respiratory effects differs from that for analgesia, or that the assessment of respiratory effects is more sensitive than the methods used for estimating analgesia.

The peak effects of R 39209 on respiratory frequency and minute volume occurred 1–2 min before those of fentanyl and its duration of action was less than half that of fentanyl. With these two exceptions, the respiratory effects of R 39209 were qualitatively indistinguishable from those of fentanyl.

Since blood PCO₂ is changed relatively slowly by respiratory depression (for example, apnoea produces an increase in PCO₂ of less than 0.5 kPa min⁻¹ (Frumin, Epstein and Cohen, 1959; Eger and Severinghaus, 1961)), the changes in PCO₂ were found to be less helpful than the changes in minute volume and respiratory frequency when examining the respiratory effects of ultra short-acting drugs.

The peak effects of fentanyl or R 39209 were remarkably reproducible as regards maximum effects. However, with successive doses of these drugs, there was a tendency for the control values not to be regained within the 10- or 15-min period (fig. 4, table III). This could indicate cumulation, but it is possible also that the drugs possess sedative effects which reduced the excitability of the rabbits. In this connection, it was observed that the rabbits given R 39209 in doses of 6 μg kg⁻¹ and more were more tranquil than those given either saline or fentanyl. Only rarely did they attempt to wriggle free of the mask, an activity which was frequent in saline-treated rabbits. An association between R 39209 and tranquillity in the period after surgery was noted during clinical studies (De Castro, 1977).

This investigation has shown that R 39209 can be given safely to spontaneously breathing rabbits. In view of its evanescent effect, it may have clinical advantages for spontaneously breathing humans who are undergoing transient yet acutely painful procedures such as manipulation of fractures, dilatation and curettage and conservation dentistry. This study in rabbits has shown also that repeated injections may be given with reproducible effects. However, the time-course must first be evaluated in man and safe time intervals for repeated injections determined.
Note added in proof: R 39209 is now known as alfentanyl.

ACKNOWLEDGEMENTS
The authors are indebted to Miss Stephanie Maddison for invaluable technical assistance and to Janssen Pharmaceutical Limited for supplies of R 39209 and some financial assistance with this project.

REFERENCES

EFFETS RESPIRATOIRES D’UN NOUVEL ANALGESIQUE OPIACE: LE R 39209, SUR LE LAPIN: COMPARAISON AVEC LE FENTANYL

RESUME
On a fait sur des lapins une étude sur les effets respiratoires d’un nouvel analgésique de courte durée, R 39209, et on a comparé les résultats obtenus avec ceux du fentanyl. On a mesuré le débit cardiaque, la fréquence respiratoire, le pH, la pCO2, et le bicarbonate du sang veineux artériel. R 39209 a eu un effet de pointe plus rapide et de plus courte durée que le fentanyl, mais

autrement les deux médicaments ont eu des effets respiratoires similaires. Le fentanyl a été entre 2 et 3,5 fois plus actif que le R 39209. Des doses répétées de R 39209 ont causé des effets de pointe reproductibles, même lorsqu’on n’a laissé que 10 min entre chaque administration.

RESPIRATORISCHE EFFEKE DES NEUEN, OPIUMHALTIGEN ANALGESIEMITTELS R 39209 IM KANINCHEN: VERGLEICH MIT FENTANYL

ZUSAMMENFASSUNG

EFFECTOS RESPIRATORIOS DE UN NUEVO OPIOATO ANALGESICO, R 39209, EN EL CONEJO: COMPARACION CON EL FENTANIL

SUMARIO
Se estudiaron en el conejo los efectos respiratorios del R 39209, un nuevo analgésico de corta duración, y se compararon con los del fentanilo. Se midió el volumen por minuto, la frecuencia respiratoria y el pH, el Pco2 y el bicarbonato típico existente en la sangre venosa oxigenada. El R 39209 mostró un efecto de cresta más temprano y una acción de menor duración que el fentanilo, pero en lo demás los efectos respiratorios de ambas drogas fueron similares. El fentanilo fue de 2 a 3,5 veces más potente que el R 39209. Repetidas dosis de R 39209 produjeron efectos de cresta reproductibles, incluso cuando se dejó transcurrir un lapsus de tan sólo 10 min entre sucesivas administraciones.