RELATIONSHIP BETWEEN ANALGESIA AND RESPIRATORY DEPRESSION FOR MU OPIOID RECEPTOR AGONISTS IN MICE

D. G. STOTT AND B. J. PLEUVRY

SUMMARY

The relationship between analgesic activity, measured as the hot plate reaction time, and respiratory depression, measured as ventilatory frequency, was investigated in mice for a variety of mu opioid receptor agonists with differing selectivities for mu receptors compared with delta receptors. There was a weak correlation between analgesia and respiratory depression for opioids with the greatest selectivity for mu opioid receptors compared with delta receptors, such as alfentanil. The strength of the correlation increased for opioids which had greater delta receptor activity, such as morphine and fentanyl. Etorphine, which has almost equal affinity for mu, delta and, incidentally, kappa receptors, showed a strong correlation between analgesia and respiratory depression. We conclude that the predictability of the degree of respiratory depression produced by a given analgesic dose of an opioid appears to decrease with its selectivity for mu opioid receptors, at least in the mouse.

KEY WORDS


For many years, standard textbooks have reiterated the observation that, for a given analgesic effect, most opioids produced the same degree of respiratory depression as seen with morphine [1]. The possibility that this need not always be the case emerged from the work of McGilliard and Takemori, who suggested that opioid analgesia and respiratory depression might be mediated by different opioid receptors [2]. Although their evidence, using naloxone antagonism, aroused considerable discussion as to the relevance of pA2 values in vivo (pA2 being a measure of antagonism normally applied to the in vitro situation), it prompted others to continue this line of investigation. Using selective irreversible mu receptor antagonists, two groups of workers [3, 4] were able to demonstrate that respiratory depression was not antagonized by mu receptor block in the unstressed rodent. One of the compounds used was naloxonazine, which Pasternak's group had reported was selective for a high affinity subgroup of mu receptors designated mu 1. Thus Ling and colleagues [3] concluded that mu 1 receptors were not involved with respiratory depression. Other workers have been unable to demonstrate mu isoreceptors with selectivity for analgesia [5]. The situation is complicated further by the fact that Ward and Takemori [4] noted that an irreversible mu antagonist could antagonize opioid respiratory depression if more stressful methods of assessing ventilation were used. The possibility that delta receptors are involved in opioid-induced respiratory depression has arisen from the observation that delta receptor antagonists may antagonize fentanyl-induced respiratory depression, but not analgesia, in the dog [6].

In the present study, the relationship between analgesia and respiratory depression has been investigated in a series of opioids with significant affinity for mu opioid receptors, but with differing selectivities for mu compared with delta receptors, ranging from alfentanil (602 (SEM 52)) and sufentanil (24.9 (1.9)) [7] to etorphine which has similar affinity for mu, delta and kappa receptors [8]. Relatively stress free measures of analgesia (or antinociceptive activity) and respiratory depres-
BRITISH JOURNAL OF ANAESTHESIA

TABLE I. Time intervals between consecutive readings of ventilatory frequency and hot plate reaction times, and drug doses used

<table>
<thead>
<tr>
<th>Interval (min)</th>
<th>Fentanyl</th>
<th>Sufentanil</th>
<th>Alfentanil</th>
<th>Morphine</th>
<th>Etorphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doses</td>
<td></td>
<td>(µg kg⁻¹)</td>
<td>(µg kg⁻¹)</td>
<td>(µg kg⁻¹)</td>
<td>(µg kg⁻¹)</td>
</tr>
<tr>
<td>4</td>
<td>0.025</td>
<td>10.0</td>
<td>0.125</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>0.05</td>
<td>20.0</td>
<td>0.25</td>
<td>5.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>40.0</td>
<td>0.5</td>
<td>10.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td></td>
<td>0.6</td>
<td></td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>0.4</td>
<td></td>
<td>0.8</td>
<td></td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.0</td>
</tr>
</tbody>
</table>

sion have been used in mice to reduce the influence of stress on the results obtained.

MATERIALS AND METHODS

MF1 mice weighing 25–30 g were used in the study, which was approved by the Home Office under the Animals (Scientific Procedures) Act 1986 (licence number 50/00499). Each group consisted of 12 MF1 male mice, which received an i.p. injection of an opioid (fentanyl, alfentanil, sufentanil, morphine or etorphine) on an mg kg⁻¹ basis in a dose volume of not more than 0.1 ml per 10 g body weight. Control values of ventilatory frequency and hot plate reaction times were recorded before any injections were given. The subsequent time intervals between the recordings, and the doses of each drug are given in table I. Each group of 12, concurrently tested, mice were allocated randomly to saline controls or one of the several doses of an individual opioid. Thus only one or two mice in the group might receive a particular dose of drug. Experiments were repeated until group sizes of 12 were obtained for each dose of drug. A saline time-matched control was available, therefore, for each opioid. Different opioids were not randomized between the groups because of the differing time courses of the experiments.

Respiratory depression was measured in terms of ventilatory frequency by recording respiratory pressure waveforms for each ventilatory cycle on a Grass 79C polygraph recorder. This was done by gently hand-holding the mouse and placing its snout in the barrel of a 2-ml syringe, connected to a pressure transducer and recorder. Recordings were taken for not more than 10 s, to reduce the possibility of rebreathing carbon dioxide. The volumes of gas involved precluded the measurement of carbon dioxide, but if rebreathing had occurred it would have been seen as an increase in ventilatory frequency and effort.

Analgesia was measured after ventilatory frequency by placing the mouse on a hot plate maintained at 55 °C and recording the time interval until the mouse showed a response—either jumping or lifting a hind leg off the plate. This was taken as an indication of analgesia. In order to prevent any tissue damage, mice were removed from the hot plate after 45 s without response. Readings for analgesia and respiratory depression were taken at suitable intervals, which varied according to the time course of action of the drugs, which had been determined by prior experimentation. With the exception of control mice, readings were continued until the hot plate reaction times had returned to pre-drug values. In time-effect relationships, the results from all drug treated mice were compared with concurrently tested vehicle control mice.

Significance (P < 0.05) was assessed using the Mann–Whitney U test although, for clarity, mean values (SE) are shown in the figures. Correlations were sought only on paired values of hot plate reaction times and ventilatory frequency changes that demonstrated significant increases in reaction time over concurrently tested saline control mice. In addition, all pairs of values containing hot plate reaction times which were greater than 45 s were excluded from calculations of the correlation coefficients.

Fentanyl, sufentanil and alfentanil were diluted in saline from prepared ampoules (donated by Janssen Pharmaceuticals Ltd). Morphine hydrochloride (Macfarlane Smith Ltd) and etorphine hydrochloride (a gift from Reckitt and Colman Ltd) were prepared by dissolving the dry powder in saline.
RESULTS

In the fentanyl and alfentanil groups, there was rapid onset of profound analgesia and respiratory depression which was relatively short lived and dose dependent. The time–response relationships for alfentanil are shown in figure 1. Although alfentanil produced significant analgesia and respiratory depression, there was no correlation between these two variables (fig. 2). A similar correlation was obtained in saline control animals, which showed no significant changes from pre-drug hot plate reaction times and ventilatory frequencies (fig. 3). At the other extreme were the results for etorphine, for which there was a strong correlation between analgesia and respiratory depression (fig. 4), although the shapes of the time–effect relationships and the maximum responses obtained (fig. 5) were similar for those recorded for alfentanil (fig. 1). Intermediate
FIG. 4. Time course of the effects of etorphine on hot plate reaction time (HPRT) and ventilatory frequency (f) in mice measured as change from pre-drug control values. Mean values (n = 12) of mice given saline (□) or etorphine 2 µg kg\(^{-1}\) (△), 4 µg kg\(^{-1}\) (▲), 5 µg kg\(^{-1}\) (○), 8 µg kg\(^{-1}\) (●), 10 µg kg\(^{-1}\) (■) and 15 µg kg\(^{-1}\) (□). A few representative SE are shown.

TABLE II. Correlation coefficients between ventilatory frequency and hot plate reaction time, and mu over delta selectivity for some opioid drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Correlation coefficient (%)</th>
<th>Mu selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etorphine</td>
<td>76.9</td>
<td>0.5 [9]</td>
</tr>
<tr>
<td>Sufentanil</td>
<td>54.2</td>
<td>24.9 [7]</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>52.4</td>
<td>82.5 [7]</td>
</tr>
<tr>
<td>Morphine</td>
<td>38.9</td>
<td>122.0 [7]</td>
</tr>
<tr>
<td>Alfentanil</td>
<td>0.3</td>
<td>602.0 [7]</td>
</tr>
</tbody>
</table>

FIG. 5. Etorphine treated mice. Relationship between change in hot plate reaction time (HPRT) and ventilatory frequency (f) depression, showing a strong simple linear correlation \(y = -20.018 - 1.8735x; r^2 = 0.769\).

correlations were obtained for sufentanil, fentanyl and morphine. The correlation coefficients for these and other opioids, together with their mu–delta selectivity are shown in table II.

**DISCUSSION**

Etorphine, which showed the strongest correlation between analgesia and respiratory depression, demonstrates a relative affinity for mu, delta and kappa receptors of 0.2, 0.4 and 1.0, respectively [9]. This implies that etorphine has little selectivity for any one of the opioid receptors; indeed, it has been termed the universal ligand for opioid receptors [8].

Alfentanil, however, which is highly selective for the mu receptor, showed a weak correlation between analgesia and respiratory depression (0.3 %). In the opioids tested in this study, weaker correlations appeared to be associated with higher mu receptor selectivity.

Most of the values for selectivity for the mu site (table II) came from a study in which only mu and delta receptor binding were considered. Thus it may be that kappa binding could influence the results. However, kappa receptor agonists are usually considered to be inactive when radiant heat, such as the hot plate, is used as the noxious stimulus [10]. Although Shaw, Rourke and Burns [11] have disputed the suggestion that various receptor-selective agonists have specificity for a particular modality of pain, they agree that kappa
agonists show little activity when the hot plate temperature is as great as 55 °C. In addition, of the three main opioid receptor types, there is least evidence that kappa receptors are involved in respiratory depression, although a modulatory role can not be excluded [12]. This evidence gives some support for concentrating on mu and delta receptor activity to explain the results obtained in the present study.

It has been suggested that mu and delta receptors may exist in two forms, either complexed together or separate [13, 14]. Both mu and delta receptor activation appear to produce analgesia at the supraspinal [15] and spinal levels [16] and activation of delta receptors enhances the response of activation of mu receptors in the complexed receptor [17]. Involvement of the mu–delta receptor complex may be important also in the control of ventilation, as there is evidence that mu receptor activation is necessary before delta receptor activation leads to respiratory depression [6]. Thus it may be that drugs with significant mu and delta activity act preferentially at the mu–delta receptor complex where the two activities cause enhanced analgesia and respiratory depression. More selective mu agonists may act either at the complexed or non-complexed mu receptor. The latter may be identical to the mu 1 receptor [13] which has been associated by some with analgesia, but not respiratory depression [3]. If this is the case, the relationship between analgesia and respiratory depression for these agents may be less predictable.

It is possible, however, that other factors contribute to the correlations obtained. Opioid drugs differ in their lipid solubility, degree of ionization at physiological pH and protein binding, all of which contribute to pharmacokinetic differences which influence their potency and duration of action [18]. Morphine, alfentanil and fentanyl have been compared with respect to these characteristics [18] and, although the rank order of individual measures differs from those of the correlation coefficients described in the present study, combinations of these properties could influence the results obtained.

REFERENCES