

## Effects of Age on Plasma Protein Binding of Sufentanil

Claude Meistelman, M.D.,\* Dan Benhamou, M.D.,† Jérôme Barre, Ph.D.,‡ Jean-Claude Levron, Ph.D.,§  
Véronique Mahe, M.D.,¶ Xavier Mazoit, M.D.,¶ Claude Ecoffey, M.D.\*\*

The plasma protein binding of sufentanil has been studied in newborns, infants ( $0.5 \pm 0.3$  yr), children ( $6.8 \pm 3.0$  yr), and adults ( $39.5 \pm 9.0$  yr). Binding of sufentanil was determined *in vitro* by equilibrium dialysis, and radioactive tritiated sufentanil was used for the determination of drug concentrations in plasma and buffer. The free fraction of sufentanil was significantly higher in the newborn ( $19.5 \pm 2.7\%$ ;  $P < 0.01$ ) than in the other age groups. The free fraction was also significantly higher in infants ( $11.5 \pm 3.2\%$ ;  $P < 0.01$ ) than in children ( $8.1 \pm 1.4\%$ ) or in adults ( $7.8 \pm 1.5\%$ ) but did not differ significantly between children and adults. The free fraction of sufentanil was strongly correlated with the  $\alpha_1$ -acid glycoprotein plasma concentration ( $r = -0.73$ ;  $P < 0.001$ ) whereas it was weakly correlated with albumin plasma concentration ( $r = -0.35$ ;  $P < 0.05$ ). These data suggest that the lower concentration of  $\alpha_1$ -acid glycoprotein in newborns and infants probably accounts for the decrease in protein binding of sufentanil in these age groups when compared with that in older children or adults. The increased free fraction in the neonate might contribute to the enhanced effects of lipophilic opioids in the neonate. (Key words: Age factors; protein binding. Analgesics: sufentanil. Anesthetics, intravenous: sufentanil. Pharmacokinetics: sufentanil.)

FENTANYL or its derivatives produce anesthesia and respiratory depression at significantly lower doses in neonates than in adults.<sup>1-4</sup> This sensitivity could result from age-related differences in either pharmacokinetics or pharmacodynamics. Because it is known that protein binding varies significantly with age, we thought that this might provide an explanation for the sensitivity of younger patients to sufentanil which is highly bound to  $\alpha_1$ -acid glycoprotein (AAG) in plasma.<sup>5</sup> Therefore the purpose of our study was to determine the effects of age on plasma protein binding of sufentanil and to relate these findings to plasma concentration of AAG which is usually lower in neonates<sup>6</sup> and also in infants<sup>7</sup> than in older children or adults.

### Methods

This study was approved by our local ethical committee and informed consent was obtained from the parents of the pediatric patients and from the adults studied. Eighteen neonates at term (38-41 weeks gestation), nine infants (2-12 months), 11 children (3-10 yr), and 11 adults (30-50 yr) were studied. None of the subjects were receiving any medication or had any disease known to modify plasma concentration of AAG or albumin. With the exception of newborn infants, venous blood samples were drawn from a peripheral vein during the preoperative period. Neonatal blood samples were drawn from the maternal end of the umbilical cord immediately after birth but before the placenta was delivered, as previously described by Wood and Wood.<sup>4</sup> Plasma was obtained by centrifugation and frozen at  $-20^\circ$  C until analysis.

All the plasma samples were studied less than 2-3 weeks after sampling. Protein binding in plasma was studied *in vitro* by equilibrium dialysis using a dianorm system through which 1 ml of plasma was dialyzed against 1 ml of a modified 1/15 molar phosphate buffer (pH: 7.4). The buffer was made isotonic with NaCl and contained 1 ng/ml of tritiated sufentanil. Its specific activity was 19.2 Ci/mmol and radiochemical purity was greater than 98% as determined by radio high performance liquid chromatography (HPLC). Before dialysis, pH of the plasma was readjusted to 7.4 with small amounts of concentrated phosphoric acid.<sup>8</sup> The cells were rotated at 20 rpm in a bath at  $37^\circ$  C. A dialysis time of 4 h was chosen, previous studies having shown that equilibrium conditions were obtained within 2-3 h. Drug concentrations in the plasma and in the buffer were determined by radioactivity measurements. Quench correction was made by the external standards ratio method.

After equilibrium had been reached, drug concentration in the buffer was equal to the concentration of free drug (concentration unbound: Cu) whereas sufentanil concentration in the plasma compartment was equal to the sum of the concentration of both free and bound drug (concentration bound: Cb). Then, the free fraction was calculated as the concentration of drug in the buffer (Cu) divided by the concentration in the plasma (Cu + Cb). Albumin and AAG were determined in duplicate by single radial immunodiffusion (Behring, Inc., Marburg, West Germany) with monospecific antiserum to human AAG and albumin in a ready for use agarose gel layer. Differ-

\* Staff Anesthesiologist, Institut Gustave-Roussy, Villejuif.

† Assistant Professor of Anesthesia, Hôpital A. Bécélère, Clamart.

‡ Pharmacologist, C.H.I.C., Créteil.

§ Pharmacologist, Janssen Laboratories, Aubervilliers.

¶ Staff Anesthesiologist, Hôpital de Bicêtre, Kremlin-Bicêtre.

\*\* Assistant Professor of Anesthesia, Hôpital de Bicêtre, Kremlin-Bicêtre.

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Address reprint requests to Dr. Meistelman: Department of Anesthesiology, Institut Gustave-Roussy, Rue Camille-Desmoulins, 94805 Villejuif Cedex, France.

TABLE 1. Free Fraction of Sufentanil, Albumin, and AAG Plasma Concentration in Different Age Groups

	Neonates (n = 18)	Infants (n = 9)	Children (n = 11)	Adults (n = 11)
Age (yr)	0	0.5 ± 0.3	6.8 ± 3.0	39.5 ± 9.0
Sufentanil (% free)	19.5 ± 2.7*	11.5 ± 3.2†	8.1 ± 1.4	7.8 ± 1.5
Albumin (g/l)	38.5 ± 3.0	37.2 ± 4.2	40.8 ± 2.7	43.9 ± 3.5†
AAG (g/l)	0.34 ± 0.07*	0.58 ± 0.11‡	0.70 ± 0.19	0.74 ± 0.12

Mean ± SD. n = number of subjects.

\* P < 0.01 neonates vs. infants, children, and adults.

† P < 0.01 infants vs. children, adults.

‡ P < 0.05 infants vs. children, adults.

§ P < 0.05 adults vs. neonates, infants, and children.

ences between groups were tested by analysis of variance (ANOVA) and Student-Newman-Keuls test. A value of  $P < 0.05$  was considered to be significant. The correlation between plasma protein concentration and free fraction of sufentanil was tested by the significance of the  $r$  value determined by least-squares linear regression. All the results are expressed as mean ± SD.

### Results

The range of pH of plasma was 7.38–7.45 at the end of dialysis. The results are summarized in table 1. Binding to the membrane was less than 3%. The free fraction of sufentanil was significantly higher in neonates than in infants ( $P < 0.01$ ), children ( $P < 0.01$ ), or adults ( $P < 0.01$ ). The free fraction was also significantly higher in infants than in children ( $P < 0.01$ ) or in adults ( $P < 0.01$ ), but did not differ significantly between children and adults. The plasma albumin concentration was slightly higher in adults than in the other age groups ( $P < 0.05$ ). The plasma AAG concentration was lower in the newborn than in infants ( $P < 0.01$ ), children ( $P < 0.01$ ), or adults ( $P < 0.01$ ). The plasma AAG concentration was also significantly lower in infants than in children ( $P < 0.05$ ) or in adults ( $P < 0.05$ ). No significant differences were observed between children and adults.

The free fraction of sufentanil was strongly correlated with the plasma AAG concentration ( $r = -0.73$ ;  $P < 0.001$ ; fig. 1). The free fraction of sufentanil was weakly correlated with the plasma albumin concentration ( $r = -0.35$ ;  $P < 0.05$ ).

### Discussion

In our study we demonstrate that the free fraction of sufentanil is increased in neonates and in infants compared with that in older children or adults. Since sufentanil is highly bound to AAG in plasma, it is likely that the lower concentrations of AAG observed in newborn and infants account for the decrease in protein binding of sufentanil in these age groups. Moreover the demonstration of a strong correlation between the plasma AAG concentra-

tion and the free fraction of sufentanil in plasma suggests that variations of the free fraction closely follow changes in plasma AAG levels. The free fraction of sufentanil does not differ between children and adults similar to that previously described with alfentanil,<sup>9</sup> and the concentration of AAG is comparable in these two groups.

The role of albumin must be questioned because plasma concentration of albumin is reduced in newborns and infants when compared with that in adults. Meuldermans *et al.* demonstrated that sufentanil exhibited only moderate affinity for albumin and was bound to AAG with greater affinity.<sup>5</sup> The plasma binding of acidic drugs such as warfarin or diazepam is dependent on the plasma concentration of albumin.<sup>5,10</sup> The correlation between the plasma concentration of albumin and the free fraction of sufentanil is weak when compared with the correlation observed between the AAG concentration and the unbound sufentanil. Furthermore, protein binding does not differ between children and adults, whereas the plasma albumin concentration is significantly higher in adults than in children. For these reasons it is less likely that the lower

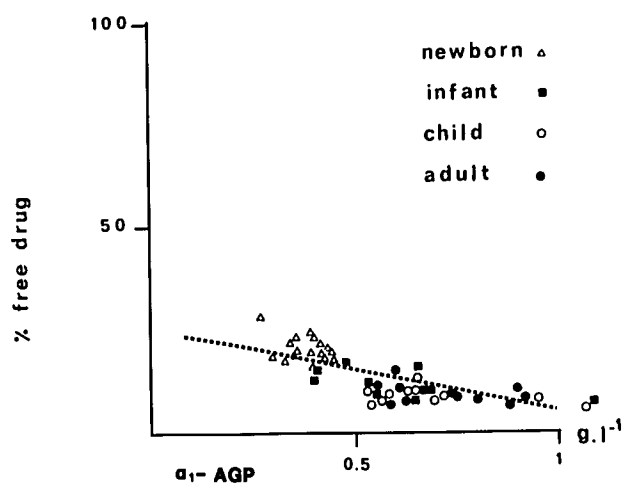


FIG. 1. Relationship between plasma  $\alpha_1$ -acid glycoprotein concentration and free fraction of sufentanil in different age groups ( $r = -0.73$ ;  $P < 0.001$ ).

level of albumin in neonates and infants is responsible for the increased free fraction of sufentanil in these age groups.

Protein binding of fentanyl and its derivatives is *pH* dependent, decreasing with increasing *pH*. Increasing the *pH* from 7.4 to 7.8 causes a 28% decrease of the free fraction.<sup>5</sup> On the other hand, at a *pH* of 7 the free fraction is increased by 29%. Even if protein binding of sufentanil is less *pH* dependant than that of fentanyl *pH* must be kept close to 7.4. Thus to obtain reproducible results for the free fraction of sufentanil the plasma samples were studied within 2 weeks to avoid a significant increase in *pH* and the *pH* of samples was brought back to 7.4 just before dialysis as previously described.<sup>8</sup> The buffer was adapted to hold the *pH* during dialysis to prevent variations of binding values. Moreover our results in adults are consistent with those of Meuldermans *et al.* who reported a free fraction of sufentanil of 7.5% in human plasma at *pH* 7.4.<sup>5</sup>

These results observed with sufentanil are comparable with those obtained with other basic drugs highly bound to AAG in plasma.<sup>6,11,12</sup> These age-related differences may have clinical implications. Yaster previously demonstrated that fentanyl produced anesthesia in neonates at significantly lower doses than in adults.<sup>1</sup> Furthermore, respiratory depression following administration of opioids occurs at significantly lower doses or plasma concentrations in neonates than in adults.<sup>1-3,13</sup> These facts have been previously attributed to an enhanced permeability of the blood-brain barrier for morphine.<sup>14</sup> This hypothesis is unlikely for fentanyl and its derivatives alfentanil and sufentanil because, in contrast to morphine, they are lipophilic and highly bound to plasma proteins.

The free fraction of sufentanil may be an important determinant of brain uptake because it is considered that the free drug can penetrate membranes and is available for entry into peripheral tissues such as the heart and brain.<sup>15,16</sup> More recently Pardridge *et al.* described transport through the blood-brain barrier for several acidic and basic protein-bound drugs.<sup>17,18</sup> Nevertheless, taking into account the dissociation constant of the protein-drug complex before entering the brain, mathematical modeling of brain uptake shows that brain extraction is correlated significantly with the unbound fraction in plasma.<sup>19,20</sup> This is why the degree of protein binding seems to be an important factor in determining uptake by the brain. Furthermore, even if it is not demonstrated that the opioid effect is directly proportional to the degree of free fraction, Lemmens *et al.* have shown in humans that the  $Cp_{50}$  of alfentanil (the total plasma concentration for which the probability of no response during surgery is 50%) was significantly correlated with its free fraction in plasma.<sup>21</sup>

Therefore, we estimate that the increased free fraction of sufentanil (143% and 47% in neonates and infants, respectively, when compared with adults) might explain the increased effects of sufentanil in the first year of life, because more drug is available to cross the blood-brain barrier, whatever its permeability. Greeley *et al.* have demonstrated that the  $Vd_{ss}$  of sufentanil was significantly more important in neonates than in children or adults.<sup>4</sup> The increase of the free fraction demonstrated in our study might contribute to the observed increase in sufentanil  $Vd_{ss}$  in neonates. Theoretically the higher free fraction may allow a more rapid redistribution from the brain into other organs but the consequences have never been investigated, the duration of action of opioids being usually longer in neonates than in adults.<sup>1,3</sup>

In children, the plasma AAG concentration may sometimes be increased significantly when compared with that in adults.<sup>7</sup> This may be due to the higher incidence of infections in children, each child having an average of three to eight episodes of upper respiratory disease each year.<sup>22</sup> Due to the inverse relationship between the plasma AAG level and the free fraction of sufentanil, the binding of sufentanil could vary widely with time in the same individual, particularly in children who are exposed to infections and changes in AAG concentration.

In conclusion, because sufentanil is highly bound to AAG, plasma-protein binding of sufentanil is highly dependent on the plasma AAG concentration. Since the plasma concentration of AAG is lower in newborns and infants, the free fraction of sufentanil, an important factor in drug distribution, is significantly higher in the early months of life.

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