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Fentanyl Concentrations in Brain and Serum during Respiratory Acid–Base Changes in the Dog

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It is a clinical impression that less fentanyl is needed for anesthesia during hyperventilation and hypocarbia. If true, it might be due to both increased penetration of fentanyl, a highly lipid-soluble agent, into the brain and increased brain tissue binding. Serum and brain concentrations of fentanyl were determined in dogs anesthetized with halothane during normocarbia, hypocarbia by hyperventilation, and hypercarbia by addition of CO_2 to the inspired mixture. Fentanyl, 12.5 μg/kg, was injected iv, and serum and brain samples were taken for fentanyl analysis by radioimmunoassay. Brain fentanyl values peaked latest (15–20 min) and were highest during hypocarbia; brain fentanyl values peaked earliest (0–5 min) and were lowest during hypercarbia; values during normocarbia were intermediate in time to peak (10–15 min) and concentration. Thereafter, brain levels declined, but during hypocarbia were significantly higher and during hypercarbia were significantly lower than during normocarbia. Interestingly, serum fentanyl levels were also significantly higher during hypocarbia. The brain–blood fentanyl ratios for each of the three CO_2 levels increased for 30 min and thereafter stayed relatively constant. The brain–blood ratios were highest with hypocarbia and lowest with hypercarbia. At 35 min, when clinical analgesia may be considered terminated, hypocarbic brain levels were double those of normocarbia. The authors feel this reflects, to a large extent, higher serum fentanyl concentrations and delayed cerebral wash-out because of decreased blood flow. To a small but unknown extent the higher brain fentanyl levels result from increased brain–blood penetration due to increased lipid solubility, and increased brain tissue binding of fentanyl during respiratory alkalosis. (Key words: Acid–base equilibrium: acidosis, respiratory; alkalosis, respiratory. Analgesics, narcotic: fentanyl. Anesthetics, intravenous: fentanyl. Brain: blood–brain barrier; carbon dioxide tension, *p*H. Solubility: blood–brain.)

CLINICAL OBSERVATION suggests that less fentanyl is needed for clinical anesthesia during hypocarbia when compared with normocarbia. Eisele, Eger, and Muallem[1] found that neither hypocarbia nor hypercarbia influenced minimum anesthetic concentration for halothane anesthesia in dogs. However, respiratory alkalosis may substantially alter the physicochemical property of fentanyl, in particular, lipid solubility. Fentanyl is a basic drug (pK_a in methanol at 25° C is about 7.5), and an increase in *p*H shifts the equilibrium to favor the unionized moiety. This makes fentanyl more lipid-soluble, which enhances penetrance of the blood–brain barrier, and in addition it increases nonspecific brain tissue binding. The reverse may be true of respiratory acidosis. Further, the effect of CO_2 on cerebral blood flow alters the rate of equilibration and hence influences the disposition of any drug. We investigated the effects of hypercarbia, normocarbia and hypocarbia on brain and serum fentanyl levels in dogs.

Methods

Mongrel dogs weighing 15–25 kg were anesthetized with thiopental, 15 mg/kg, intravenously. After their tracheas were intubated anesthesia was maintained with halothane in oxygen. The dogs were subsequently paralyzed with pancuronium and their lungs ventilated with a respirator. Catheters were inserted into a femoral artery and a forepaw vein. Expired CO_2 was measured on a Beckman LB2 infrared analyzer. Transduced arterial blood pressure (Statham p23), EKG, and heart rate were continuously displayed on a Grass polygraph. Rectal temperature was measured and maintained at 36–38 C. Lactated Ringer's solution was infused at 10 ml/kg/hr, with increments for blood loss. Arterial blood-gas measurements were made at frequent intervals and corrections made when necessary. A right temporoparietal craniectomy was performed and an area of cortex large enough for ten to 12 separate sampling sites was exposed. Brain tissue temperature was measured with a thermistor probe.

The dogs were divided into three groups of six animals each. Group I was kept normocarbic (Pa_{CO_2} 36 torr). Group II was made hypocarbic by hyperventilation (Pa_{CO_2} 19 torr). Group III was made hypercarbic by addition of CO_2 to the inspired mixture (Pa_{CO_2} 65 torr). An equilibration period of 30 min was allowed prior to fentanyl administration and brain sampling.

Atropine, 0.2 mg/kg, was given intravenously to decrease the incidence and severity of bradycardia. Fentanyl citrate, 12.5 μg/kg, was given intravenously over one minute, and 5-ml blood samples were drawn

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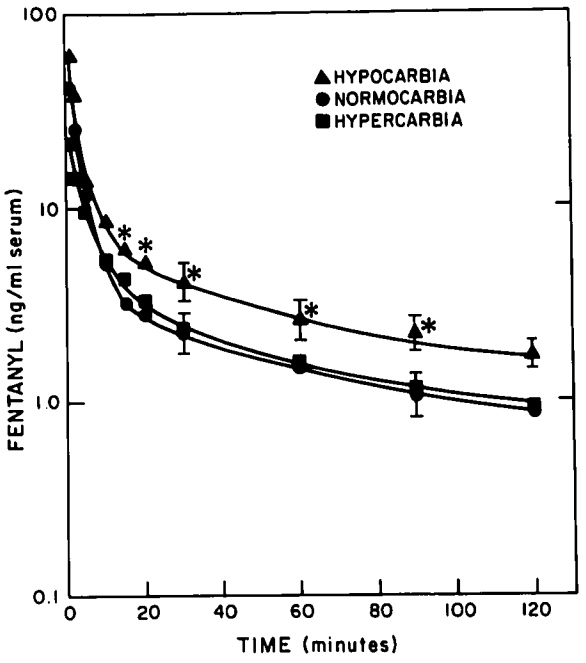

FIG. 1. Fentanyl levels in serum measured over two hours in three groups of dogs: normo-, hypo-, and hypercarbia. Asterisks indicate significant differences when compared with the normocarbia points by the Student *t* test. *$P < .05$.

at 1, 2, 5, 10, 15, 30, 60, 90 and 120 min. The serum was separated and frozen until analyzed. Approximately .05-g samples of cerebral cortex, 2–4 mm in depth, were taken with a sharp instrument, after which hemostasis was achieved with microfibrillar collagen. Samples were taken at 5, 10, 20, 30, 60, 90, and 120 min. Attempts were made to sample only areas undisturbed by prior biopsy. The brain tissue consisted of mixed grey and white matter. Samples were rolled on blotting paper, and after removal of any blood a wet weight recorded. A 2-ml volume of borate buffer solution was added and the mixture was ground to a uniform consistency. The homogenate was pipetted, frozen and stored for analysis. Cortical homogenates and sera were analyzed for fentanyl in duplicate by radioimmunoassay. The method detects both bound and free fentanyl and is sensitive to 300 pg/ml.[2]

Each point in the time concentration curves represents a mean of six dogs in Groups I, II, and III (figs. 1 and 2). Serum and brain concentrations of fentanyl during hypocarbia and hypercarbia at each respective sampling point were compared with the normocarbia values by Student's *t* test, for unpaired data. $P < 0.05$ was regarded as significant. Brain–blood ratios were calculated at various intervals. After logarithmic conversion of the fentanyl data, time–concentration curves were plotted. Except for the initial brain distribution, the half lives ($t_{1/2}$) of the fast and slow phases were calculated by the exponential peeling logarithms–linear regression method (tables 1 and 2). Hypocarbia and hypercarbia ($t_{1/2}$) values were compared with the normocarbia ($t_{1/2}$) value by Student's *t* test.

In two additional dogs brain fentanyl kinetics were studied following pentobarbital, 30 mg/kg, for comparison with those values obtained with halothane.

Results

Heart rate changes following fentanyl administration were eliminated by atropine. The mean maximal decrease in arterial blood pressure was 15 per cent. Ventilatory adjustments and increased fluids were necessary to compensate for increased physiologic dead space following atropine and the decrease in blood pressure. Mean Pa_{CO_2} values for each group were 19 ± 0.2 torr during hypocarbia, 40 ± 0.5 torr during normocarbia, and 65 ± 0.6 torr during hypocarbia. Pa_{O_2} remained above 450 torr in each group, and there was no metabolic derangement.

Serum concentrations of fentanyl 1 min following injection during hypocarbia were 61 ± 12 ng/ml, during normocarbia, 43 ± 7 ng/ml, and during hypercarbia, 21 ± 6 ng/ml (fig. 1). Fentanyl concentrations declined rapidly during the first 10 min in each group. Serum levels of fentanyl during hypocarbia were approximately double normocarbia and hypercarbia after 30 min. Wash-out curves appear to fit a triphasic

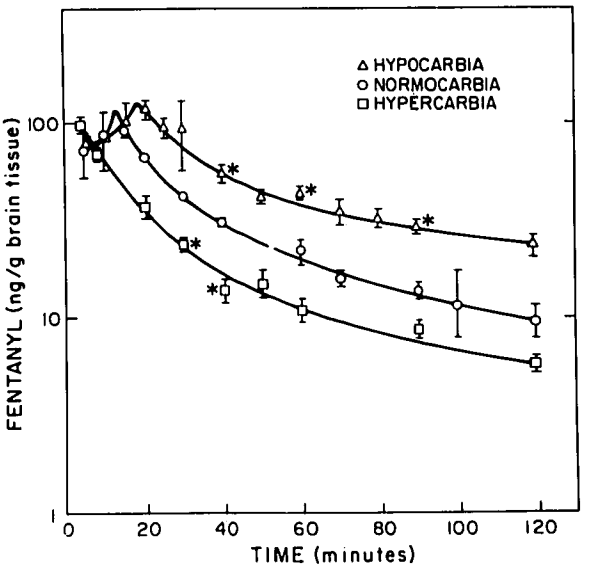

FIG. 2. Fentanyl levels in brain measured over two hours in the three groups of dogs: normo-, hypo-, and hypercarbia. Asterisks indicate significant differences when compared with the normocarbic points by the Student *t* test. *$P < .05$.

TABLE 1. Serum Kinetics*

	Normocarbia	Hypocarbia	Hypercarbia
$t_{1/2}$ (min) fast	17 ± 2	21 ± 2	20 ± 3
$t_{1/2}$ (min) slow	89 ± 14	80 ± 10	80 ± 10

* Serum half times for fentanyl wash-out. Fast phase was taken between 10 and 30 min and the slow phase was taken between 40 and 120 min. This was done to correlate serum with brain kinetics.

TABLE 2. Brain–Blood Fentanyl Concentration Ratios*

Time (Min)	Normocarbia	Hypocarbia	Hypercarbia
5	7	4	14
30	18 ± 2	17 ± 1	11 ± 2†
60	13 ± 2	18 ± 1‡	7 ± .6†
90	13 ± 1	15 ± 1	7 ± .7†
120	9 ± 2	14 ± 2	6 ± .2†

* Ratios of brain–blood fentanyl concentration at corresponding times for the three groups.
† $P < .05$ compared with normocarbia and hypocarbia.
‡ $P < .05$ compared with normocarbia.

pattern. However, to compare with the brain wash-out curve we arbitrarily partitioned the serum curve into two components, a rapid phase from 10 to 30 min and a slow phase from 40 to 120 min. Half lives for both the fast and slow phases were similar in the three groups (table 1).

At all sampling times and each Pa_{CO_2} level, brain fentanyl concentrations exceeded the serum concentrations. The brain–blood ratio after 5 min during hypocarbia was 4.4, during normocarbia, 7.1, and during hypercarbia, 13.7 (table 2). In all three groups this ratio increased for 30 min and then stayed relatively constant. After 30 min the ratio was higher for hypocarbia and significantly lower for hypercarbia compared with normocarbia. Estimated peak brain levels were higher during hypocarbia (121 ng/g) and lower during hypercarbia (98 ng/g) than during normocarbia (113 ng/g). The decrement phase was divided arbitrarily into fast and slow components, which appeared to fit the data best. Half lives ($t_{1/2}$) for the fast phase as well for the slow phase were slightly longer during hypocarbia and hypercarbia compared with normocarbia (table 3). Brain concentrations of fentanyl during hypocarbia and hypercarbia were significantly higher and lower, respectively, than normocarbic levels at some of the periods sampled. At 35 min brain levels of fentanyl with hypocarbia were double normocarbia levels, and three times hypercarbia concentrations (fig. 2).

In three dogs anesthetized with pentobarbital the mean maximal decrease in blood pressure was 30 per cent, compared with 15 per cent in the dogs anesthetized with halothane. *p*H and Pa_{CO_2} values were similar in the two groups, as were brain fentanyl values (fig. 3).

Discussion

Fentanyl pharmacokinetics have been studied in rabbits,[3,4] in man,[5] and in the dog.¶ Our data on fentanyl serum wash-out curves during normocarbia closely approximate those of Murphy after adjustment for different dosage (12.5 *vs.* 10 μg/kg). For brain fentanyl during normocarbia our data did not duplicate the decrement curves of previous studies (Hess 1971,[3] Hollt 1975[6]) due to different dosage schedules and fewer sampling points in their studies. However, our brain–blood ratio five min after injection during normocarbia was 7:1, compared with the value obtained by Hess in the rabbit of 9:1.[3] Fentanyl tissue concentrations for lung, kidney, heart and brain greatly exceed serum concentrations within ½ min following injection.[3] High tissue levels of fentanyl are a consequence of facilitated passage through biologic membranes and nonspecific tissue binding, both of which are related to the lipophilia of the drug (*n*-heptane number of fentanyl is 19 at *p*H 7.4).[6] Changes in *n*-heptane number due to *p*H changes interpolating from Hollt are 15 at 7.25 and 22 at 7.55. This indicates a 47 per cent increase in lipid solubility when alkalosis is compared with acidosis.

Increased *p*H leads to increased per cent of the unionized moiety and increased lipophilia. The pK_a for fentanyl in methanol is 7.47 at 25 C, and most authors assume a pK_a value in blood of 7.5. From the Henderson-Hasselbalch equation we calculate that the unionized portion is 44.3 per cent at normocarbia (*p*H 7.37), 56.3 per cent at hypocarbia (*p*H 7.58), and 36.5 per cent at hypercarbia (*p*H 7.23).

In our study, 1-min serum fentanyl concentrations were 43 ± 7 ng/ml during normocarbia, 61 ± 12 dur-

TABLE 3. Brain Kinetics*

	Normocarbia	Hypocarbia	Hypercarbia
Time to peak (min)	10–15	15–20	0–5
$t_{1/2}$ (min) fast	13	17	16
$t_{1/2}$ (min) slow	40 ± 9	60 ± 9	63 ± 6†

* Brain fentanyl half times for fast and slow phases of the wash-out curve, and times to peak for the three groups.
† $P < .05$ compared with normocarbia.

¶ Murphy MR, Olsen WA, Hug CC Jr: Pharmacokinetics of ^{3}H fentanyl in the dog (abstr). American Society of Anesthesiologists, Annual meeting, 1977, p. 47.

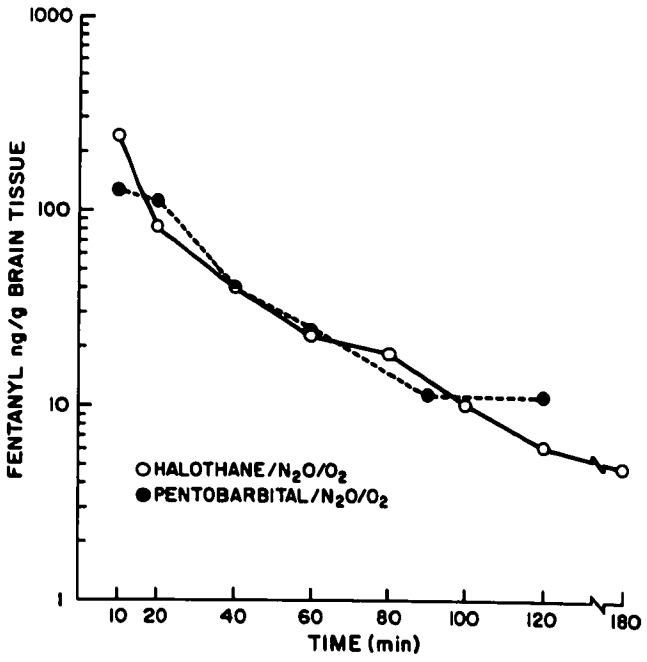

FIG. 3. Brain concentrations of fentanyl after iv injection (25 μg/kg) in two dogs, one anesthetized with halothane–N_2O–O_2 and the other with pentobarbital (30 mg/kg)–N_2O–O_2.

ing hypocarbia, and 21 ± 6 during hypercarbia. The low hypercarbia concentration probably reflects the indirect effects of CO_2, whereupon catecholamines liberated cause increased circulatory dynamics and a more rapid redistribution of drug. Of greater interest was that serum fentanyl levels during hypocarbia were twice those during normocarbia and hypercarbia from 15 min to 120 min (fig. 1). This could be due to decreased amounts of drug available to the liver for metabolism, because of a reported 22 per cent decrease in hepatic blood flow during respiratory alkalosis to *p*H 7.55.[7] It may also be due to a decreased cardiac output during controlled hypocarbia,[8] and decreased sympathetic tone,[9] both factors tending to decrease circulation and delay clearance. Protein binding of fentanyl is high (67 per cent) and is increased during alkalosis (to 76 per cent),[6] which also tends to delay metabolism. We did not expect this difference to be so large, and do not know of other studies that evaluate this point.

An early peak on the brain wash-out curve during hypercarbia (0–5 min), and a delayed peak during hypocarbia (15–20 min) can be explained by the effects of increased and decreased cerebral blood flow, respectively, on the rate of equilibration. Fentanyl levels during the wash-out phase are related to circulatory factors until a relatively steady state is reached. In this study brain–blood ratios were more or less constant after 60 min in each group (table 2). Changes in solubility and tissue binding should affect blood as well as brain, however, increased lipophilia would favor brain tissue, which has more binding sites. This might explain the somewhat higher brain–blood fentanyl ratios during hypocapnia. Altered brain–blood barrier characteristics could cause greater penetration, but to our knowledge this has been studied only during hypercarbia, wherein the barrier is impaired.[10]

Halothane was used in this study for cardiovascular stability. We were concerned about the increase in cerebral blood flow seen with halothane and the possible influence on fentanyl brain concentrations. However, brain levels and wash-out curves for fentanyl were shown to be similar for pentobarbital and halothane at the same Pa_{CO_2} (fig. 3).

No attempt was made to correlate fentanyl brain levels with clinical analgesia. Good correlation was found by Von Cube (1970)[11] in rats and by Hess (1971)[3] in rabbits between fentanyl brain levels and threshold for response to tooth-pulp stimulation. Our findings during hypercarbia were different from those of Finck *et al.* (1977).[12] We found that during hypercarbia brain levels of fentanyl were lower than those during normocarbia, whereas Finck found that with morphine, during hypercarbia, brain levels were higher than normocarbia. Compared with fentanyl, morphine is relatively lipid-insoluble (*n*-heptane number .001, compared with 19 for fentanyl), with a higher pK_a (8.0 compared with 7.5); hence, it is not surprising that the results differ.

In conclusion, we studied blood and brain decrement curves for fentanyl in the dog at Pa_{CO_2} levels of 19, 36, and 65 torr. At 35 min, which is considered the end of clinical analgesia, brain levels during hypocarbia were 68 ng/g, during normocarbia, 34 ng/g, and during hypercarbia, 21 ng/g. If an increased concentration at the stereospecific active site follows from an increased concentration at the lipophilic nonspecific site, due to the *p*H change,[13] then we postulate that at 35 min the hypocarbic dogs experienced greater analgesia than the normocarbic dogs. By extrapolating equi-brain concentrations from the normocarbic curve to the hypocarbic curve, clinical analgesia may last considerably longer in the hypocarbic dogs. The principle can probably be extended to any fixed agent that is weakly basic and highly lipid-soluble, and whose brain binding site is lipophilic.

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