Blood-Brain Barrier: Penetration of Morphine, Codeine, Heroin, and Methadone after Carotid Injection

Abstract. *Labeled morphine, codeine, heroin, or methadone was injected as a bolus into the common carotid artery of the rat, and the rat was decapitated 15 seconds later. The brain uptake of the drug was calculated by measurement of the brain content of the drug as a percentage of a labeled, highly diffusible reference substance simultaneously injected. The uptake of morphine was below measurability; the uptake of codeine was 24 percent; heroin, 68 percent; and methadone, 42 percent. Brain uptakes of morphine and codeine were also studied after intravenous injection and correlated well with uptakes after carotid injection; the uptake of codeine being nearly complete by 30 seconds. These studies indicate that brain uptake of certain of these drugs is very rapid and that uptake of heroin injected intravenously is probably limited by the regional flow of blood in the brain. The possible relation of this rapid penetration of the blood-brain barrier by heroin to its strongly addictive properties is discussed.*

The blood-brain barrier is often a major factor determining the amount of a drug that reaches the extracellular fluid of the brain after systemic administration. In the usual experimental methods of measuring the permeability of the blood-brain barrier, a radiolabeled or unlabeled test drug is introduced systemically, and its brain concentration is measured at some later time. The blood-brain barrier is considered permeable when appreciable amounts of the drug appear in the brain. Plasma protein binding, variable systemic biotransformation of the drug, and other factors, may obscure the intrinsic permeability of the blood-brain barrier to the unbound original compound. In our study we utilize a technique in which the test substance is injected quickly into the common carotid artery of the rat, thereby introducing a predictable amount of test substance into the microcirculation of the brain. The amount of test substance taken up by the brain during a single microcirculatory passage is used as an index of permeability of the blood-brain barrier.

The labeled narcotics (*1*) shown in Fig. 1 were each injected as a bolus into the surgically exposed common carotid artery of adult rats anesthetized with pentobarbital (*2*). The injection vehicle was 0.2 ml of Ringer solution (*3*). In addition to the test substance, the injected solution also contained tritiated water as a diffusible standard (*4*) when the test substance was [^{14}C]codeine, [^{14}C]morphine, or [^{14}C]heroin; [^{14}C]isopropanol was the diffusible standard when the test substance was [^{3}H]methadone. The rat was decapitated 15 seconds after the injection.

Blood to brain exchange of the test substance occurs in the first 1 to 2 seconds after injection, and mixing with blood during this time is minimized because of the high rate of bolus injection. It is assumed that nearly 100 percent of the diffusible reference substances had penetrated the blood-brain barrier and remained in the brain. Some fraction of the injected test substance also had penetrated the blood-brain barrier and remained in the brain at 15 seconds. Any of the test substance remaining intravascular was, by the time of decapitation, cleared from the vasculature and distributed to the entire rat. The amount of test substance remaining in the brain at 15 seconds after the injection was expressed as a percentage of the diffusible reference substance present at that time. This calculation was carried out after routine simultaneous ^{14}C and ^{3}H liquid scintillation counting of approximately 0.2 g of brain tissue rostral to midbrain and ipsilateral to the carotid injection.

When this study was carried out with the use of nondiffusible test substances, such as sucrose or inulin, about 2 percent remained in the brain at 15 seconds (*4*). This 2 percent is considered the background level of the method and has not been subtracted to calculate the brain uptakes reported in our study.

The brain uptakes of the drugs studied after carotid injection are shown in Fig. 1. The uptake of morphine is not significantly elevated above the 2 percent background level of the method. The uptake of codeine is 24 ± 3 percent; heroin, 68 ± 6 percent; and methadone, 42 ± 3 percent (*5*).

When measuring the brain uptake of a test drug after routine systemic administration it is common to wait some minutes after administration to determine brain concentration. Since our carotid injection studies indicate that a large fraction of codeine, heroin, and methadone penetrate into the brain during a single capillary passage, it was postulated that distribution of many drugs to the brain take place during the first few seconds after intravenous injection. A delay of some minutes before measuring brain concentration may give a spurious impression of the rate of distribution to the brain. To determine the relevance of the brain uptakes of drugs after carotid injection to the more usual systemic administration, we studied the concentrations of codeine and morphine in plasma and brain starting at 30 seconds and up to 32 minutes after intravenous administration. Heroin was not studied after intravenous injection.

Approximately 2 μc of [^{14}C]codeine, 4 μc of [^{14}C]morphine, or 4 μc of [^{3}H]mannitol (*1*) were injected into the femoral vein of Fisher rats (200 g) (*6*), which were decapitated 0.5, 1, 2, 4, 8, 16, or 32 minutes later. Radioactivity in weighed blood plasma and brain was determined by routine liquid scintillation counting immediately after decapitation. The entire brain rostral to the midbrain was included in the specimen. A portion of the injected solution was weighed and counted. The [^{3}H]mannitol was included in the study to compare the rate of its disappearance from plasma and its appearance in brain relative to codeine and morphine (*7*). The brain and plasma concentrations in this intravenous study are expressed as a percentage of the mean body concentration (*8*).

Figure 2 shows the concentration of [^{14}C]codeine and [^{14}C]morphine in plasma and brain at various times after intravenous injection. By 30 seconds after injection more than 90 percent of both codeine and morphine have left the blood plasma (*9*). The maximum concentration of brain codeine is reached by 1 to 4 minutes and is nearly at the maximum level by 30 seconds. This very rapid entry into brain correlates with the 25 percent uptake noted in the single brain passage after carotid injection (Fig. 1).

At no time during the 32-minute period did the brain concentration of morphine exceed approximately 6 percent of the mean body concentration after intravenous injection (Fig. 2). Throughout the 32-minute interval the brain concentration of morphine was approximately one-tenth that of codeine. This relation to codeine correlates well with the relative brain uptakes after carotid injection (Fig. 1).

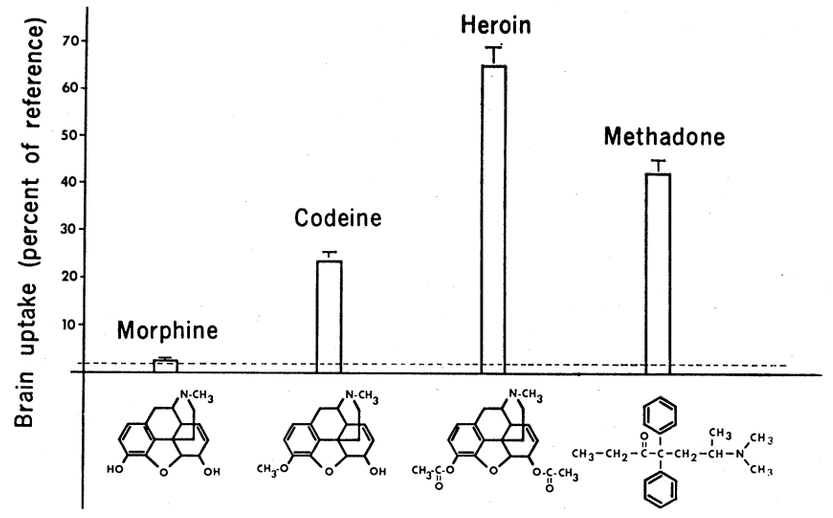

Fig. 1. Structural formulas and brain uptakes of morphine, codeine, heroin, and methadone in a single brain passage after carotid injection. The greater uptake of codeine and heroin relative to morphine can be explained on the basis of their greater lipid solubility relative to morphine. For each mean and standard deviation, n = six rats.

Although methadone and heroin were not studied after intravenous injection, it is reasonable to assume their entry into brain would be even more rapid than codeine.

Both [^{14}C]codeine and [^{14}C]morphine disappeared from plasma considerably more rapidly than did [^{3}H]-mannitol (Fig. 2). The brain concentration of [^{14}C]morphine was always higher than that of [^{3}H]mannitol.

The amount of a drug that distributes to brain after introduction into the body is a function of several unpredictable variables. Some of these are: (i) the rate of transfer into blood from the intestinal lumen or other entrance site, (ii) the fraction of circulating drug bound to plasma protein, (iii) the freedom with which the unbound circulating fraction of the drug penetrates the blood-brain barrier, (iv) the binding by the brain once the drug has penetrated the blood-brain barrier, (v) the biotransformation of the drug prior to and after penetration into brain, and (vi) the fraction of the drug ionized at blood pH. With systemic administration it is difficult to separate the penetration of the blood-brain barrier [see (iii) above] from other variables.

Mixing of the test drug with blood is minimized by the high rate of common carotid injection. After the brief period of injection, forward circulation resumes and the injected bolus passes through the brain reasonably well isolated from contact with plasma. With all systemic routes of administration, the test drug is in contact with blood protein prior to its passage through the microcirculation of the brain and, depending on the affinity of the drug for plasma protein binding sites, some variable fraction remains in free solution. Probably only this unbound plasma fraction is available for blood to brain exchange, and it presumably is the rapidity of the exchange of this unbound fraction that our carotid injection methodology defines. The injected labeled test substance is, after carotid injection, almost entirely in free solution when it enters the microcirculation of the brain where its rapidity of uptake by the brain probably is greater than the uptake that would occur if the test substance had been carried into the brain mixed with blood and thus partially protein bound. However, plasma protein binding studies of several morphine analogs (*10*) and methadone (*11*) indicate that 33 to 82 percent of these drugs are unbound in plasma. This suggests that, at least for these relatively poorly bound drugs, the brain uptakes after carotid injection (Fig. 1) can predict the rate of penetration of the blood-brain barrier after systemic administration.

Our studies indicate that some drugs come into equilibrium between blood and brain within a few seconds and that the amount of drug taken up by the brain after systemic administration is often determined by its rate of appearance in the blood, particularly after subcutaneous, intramuscular, or intraperitoneal injection. After intravenous injection the concentration in brain during the first 30 seconds is of interest with many drugs because blood or tissue sampling delayed more than a few minutes after injection may miss initially high values. Our data (Fig. 2)

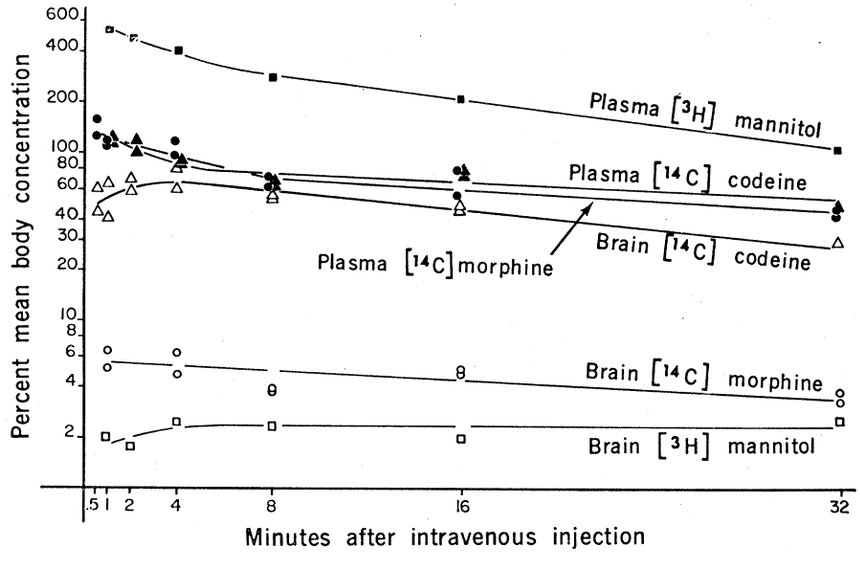

Fig. 2. Tissue concentrations of [^{14}C]-codeine and [^{14}C]morphine in brain after intravenous injection in the rat expressed as a percentage of mean body concentration. By 30 seconds, more than 90 percent of both codeine and morphine are no longer present in the blood plasma, and nearly maximum concentrations of codeine have already been achieved in the brain. The considerably lower concentrations of morphine in the brain seen here after intravenous injection correlate well with the relative uptakes of codeine and morphine after carotid injection, as shown in Fig. 1.

suggest that brain uptake studies of systemically administered drugs should, in most cases, use intravenous injection with brain tissue concentration measurements beginning at 30 seconds.

To be entirely representative of brain uptake of clinical doses of drugs, the intravenous studies described here would have to be repeated in the presence of therapeutic blood concentrations of unlabeled drug. The tracer concentrations used in our studies would probably be bound more completely to plasma protein than when present in therapeutic concentrations. If penetration of the blood-brain barrier by the drug was, in part, carrier-mediated, significant saturation effects could be seen with therapeutic concentrations but not with trace amounts.

The observation that most of the heroin entering the brain after carotid injection is taken up by the brain suggests that delivery to any region of brain tissue is largely determined by the regional flow of blood in the brain. Accordingly, after rapid intravenous injection the amount of heroin deposited regionally in the brain is probably approximately in proportion to the blood flow (*12*). Even though heroin is rapidly hydrolyzed in blood to 6-monoacetylmorphine and to morphine (*13*), it probably largely survives as heroin the 10 to 15 seconds required to be delivered peripherally after intravenous injection in humans. Once in the brain it is rapidly hydrolyzed to 6-monoacetylmorphine and to morphine (*13*). Redistribution of this morphine back through the blood-brain barrier to circulating blood probably is retarded both by tissue binding and by the low permeability of the blood-brain barrier to morphine. These kinetics may, in part, account for the greater pharmacological effectiveness of heroin.

The observation that both codeine and morphine disappear from blood plasma substantially more rapidly than mannitol (Fig. 2) is consistent with the observation that morphine has a high affinity for many general tissue binding sites (*14*).

The high uptake of heroin reported here after carotid injection indicates that an abrupt entrance of heroin into brain tissue probably occurs 10 to 20 seconds after the usual intravenous injection by addicts. This rapid entry relative to morphine may reinforce the addict's relating the act of drug administration to central nervous system response and thereby be a factor in the more intractable addiction to heroin. This rapid entry into brain may also be a factor in nicotine dependence since its brain uptake when studied by the present carotid technique is more than 100 percent (*15*).

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References and Notes

1. L-[1-^{3}H]Methadone hydrobromide (92 mc/mmole) and D-[1-^{3}H]mannitol (nominally labeled) (3140 mc/mmole) were from New England Nuclear. [*N*-methyl-^{14}C]Morphine hydrochloride (58 mc/mmole) and [*N*-methyl-^{14}C]codeine hydrochloride (48 mc/mmole) were from Amersham/Searle. [^{14}C]Morphine from Amersham/Searle was acetylated by Dhom Products to produce diacetyl morphine (heroin).
2. Rats that received carotid injections were Wistars of either sex (300 g) that were anesthetized with pentobarbital intraperitoneally.
3. Ringer solution for carotid injections contained 4 or 10 mmole of HEPES buffer (Calbiochem) per liter and was injected at a *p*H of 7.55.
4. W. H. Oldendorf, *Brain Res.* **24**, 372 (1970); *Amer. J. Physiol.* **221**, 1629 (1971).
5. It was assumed that the chemical structure of the labeled compound was unaltered between the time of injection and penetration of the blood-brain barrier and that the radionuclide which had penetrated the blood-brain barrier remained in the brain during the 15-second vascular compartment washout. Biotransformation after penetration of the blood-brain barrier was assumed to be unimportant since it would not result in a change in the amount of radionuclide remaining in brain tissue. For mean value, $n = 6$.
6. Fisher 344 rats (Charles River) weighed approximately 200 g and were not nephrectomized.
7. Mannitol is a small molecule (molecular weight, 182) not significantly bound to plasma protein, and it served as a lipid insoluble, metabolically inert reference substance that would remain largely extracellular.
8. The calculation of percent mean body concentration is: [(count min^{-1} injected per gram of tissue)/(count min^{-1} per gram of total body weight)] × 100. Brain tissue concentrations are corrected for labeled compounds present in residual brain blood plasma, assuming 0.8 percent of brain tissue is plasma.
9. If the test substance remained confined to plasma, the plasma concentration throughout the interval studied would be about 2500 percent of the mean body concentration because the plasma volume is about 4 percent of the total body weight.
10. L. B. Mellett, *Bulletin on Drug Addiction and Narcotics* (National Academy of Sciences–National Research Council, Washington, D.C., 1964), appendix 12, 3822.
11. C. D. Olsen, *Science* **176**, 525 (1972).
12. L. A. Sapirstein, *Amer. J. Physiol.* **193**, 161 (1958).
13. E. L. Way, in *The Addictive States*, A. Wickler, Ed. (Williams & Wilkins, Baltimore, 1968), p. 13; *Arch. Biol. Med. Exp.* **4**, 92 (1967); ———, J. M. Young, J. W. Kemp, *Bull. Narcotics* **17**, 25 (1965).
14. D. N. Teller, T. De Guzman, A. Lajtha, *Neurology* **22**, 414 (1972).
15. The brain uptakes of a number of other drugs have been reported by means of the carotid injection with a tritiated water diffusible internal standard [W. H. Oldendorf, *Trans. Amer. Neurol. Ass.* **96**, 46 (1971)]. These uptakes (percent) are: nicotine, 119; ethanol, 117; imipramine, 107; procaine, 91; caffeine, 86; antipyrine, 65; cyanide, 47; diphenylhydantoin, 28; phenobarbital, 18.7; mescaline, 5.6; L-ascorbic acid, 3.8; aspirin, 2.65; and benzylpenicillin, 2.1. Brain uptakes greater than 100 percent probably represent initially complete clearance of the test substance with less washout than the tritiated water diffusible reference during the 15-second washout interval.

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Antigens Specific for Human Lymphocytic and Myeloid Leukemia Cells: Detection by Nonhuman Primate Antiserums

Abstract. *Primate antiserums to human leukemia cells can detect antigens specific for lymphocytic leukemia cells or antigens present on certain myeloid leukemia cells. The antigen specific for lymphocytic leukemia cells is destroyed by treatment with neuraminidase or trypsin. Tryptic digests of lymphocytic leukemia cells contain the antigen, which has a high molecular weight.*

Immunization of monkeys with peripheral blood cells from individual patients with chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), or chronic granulocytic leukemia (CGL) leads to the production of high-titered antibodies which, after appropriate absorption techniques, appear to be detecting antigens specific for either lymphocytic leukemia or certain myeloid leukemia cells.

For immunization of primates, leukocytes were obtained from buffy coat cells from four different leukemia patients placed on an Aminco cell separator. Most of the erythrocytes were removed from the buffy coat suspension by sedimentation with Plasmagel (*1*). Leukemia cells used for the initial immunization of primates were thrice washed with Hanks balanced salt solution (> 90 percent viability) and injected on the same day. Some of the cells to be used later for booster immunizations and testing were suspended in RPMI (Roswell Park Memorial Institute) 1640 medium with 10 percent autologous serum and 10 percent dimethyl sulfoxide, frozen, and stored in liquid nitrogen. Cells for the initial injection (10^9) were emulsified in Freund's complete adjuvant (*2*) and injected intradermally and subcutaneously into various species of monkeys. Cells from one patient with CLL (HL-A1, 8, 12) were injected into a