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Morphine and other opiates from beef brain and adrenal

(opioids/hypothalamus/isolation/NMR/immunoreactive morphine)

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ABSTRACT **We describe nonpeptide opioids found in extracts of beef hypothalamus and adrenal, which are recognized by antisera raised against morphine. Four have been purified to homogeneity. One is morphine. The structures of the other three have not been determined yet. None of them are derived from morphine or normorphine after extraction from the tissues. It is not known whether the opiates described here are of endogenous or exogenous origin.**

In 1973 one of us (1) postulated the existence of an endogenous opioid in brain and attempted to detect it by immunoassay, using an antiserum raised against morphine. In mouse brain no morphine immunoreactivity (ir-morphine) could be found at a detection limit of 16 pmol per brain. In 1976, however, Gintzler *et al.* (2) reported the presence of ir-morphine in rabbit and cat brain, and similar findings have been published by this (3–7) and other (8, 9) groups from time to time. To date, no proof of structure of any of these substances from mammalian tissues has been published. However, Hazum *et al.* (10) isolated a morphine-immunoreactive compound from cow's milk, which behaved identically to morphine in three HPLC systems and in mass spectrometry. They suggested the likelihood of a dietary source and commented that they had found ir-morphine in various animal fodders.

The investigations reported here were stimulated by the conspicuous absence of an endogenous opioid with high selectivity for the μ opioid receptor (11, 12). The positive findings cited above, as well as earlier ideas of Davis and Walsh (13) about a possible endogenous biosynthesis of morphine in mammalian brain, led us to resume the search interrupted a decade earlier. We have now identified several immunoreactive morphine-like substances in beef brain and adrenal. We have purified four of them to homogeneity and determined the structure of one, which is morphine.

MATERIALS AND METHODS

Materials. Peptides were obtained from Peninsula Laboratories (Belmont, CA), Bachem Fine Chemicals (Torrance, CA), Biosearch (San Rafael, CA), or Pierce. Purity of all compounds was verified by HPLC, either by the supplier or by us; when necessary, we purified them by reversed-phase HPLC. The following were gifts: metorphamide, from E. Weber; oripavine, from A. Jacobson and E. Brochmann-Hansen; thebaine, codeinone, and reticuline, from E. Brochmann-Hansen. Various opioids and related compounds were obtained from the indicated suppliers: salsolinol, Aldrich; sufentanil, Janssen Pharmaceutica (Beerse, Belgium); normorphine and dihydromorphine, Applied Science (Waltham, MA); levorphanol and dextrorphan, Hoffmann–La Roche; morphine, S. S. Penick (Lyndhurst, NJ); tetrahydropapaveroline (norlaudanosine) and morphine 3-glucuronide, Sigma; naloxone and oxymorphone, Endo Laboratories (Garden City, NY); hydromorphone, Knoll Pharmaceutical (Whitman, NJ); codeine, Burroughs Wellcome (Research Triangle Park, NC); naltrexone, National Institute on Drug Abuse; [^{3}H]morphine (methyl-labeled), New England Nuclear; [^{3}H]morphine (ring-labeled) and Na^{125}I, Amersham. Other reagents were purchased from Baker or Sigma.

Radioimmunoassay (RIA). Antisera to morphine were generously donated by Syva (Palo Alto, CA). After screening them, we selected two (both from sheep) for sensitivity to morphine and differing specificities toward various morphine congeners. Both antisera were furnished as 50% ammonium sulfate fractions (50–100 mg/ml). Antiserum 937 was raised using as immunogen morphine conjugated to bovine gamma globulin at position 3 through an ethylamine linker. For antiserum S17, conjugation was through carboxymethyl to the N atom of normorphine. Morphine was iodinated by the chloramine-T procedure (14) and then applied to a Sep-Pak cartridge (Waters Associates), and eluted with a 1:1 (vol/vol) mixture of acetonitrile and 10 mM CF_3COOH. Final purification of ^{125}I-labeled and ^{3}H-labeled tracers was by reversed-phase HPLC in the system described below. Stock solutions were stored at −20°C in 0.1 M HCl with 1% 2-mercaptoethanol.

Dilutions were in 150 mM phosphate buffer (pH 7.4) containing 0.1% bovine serum albumin and 0.1% Triton X-100. An assay tube contained 100 μl of diluted sample, 100 μl of diluted antiserum, and 100 μl of ^{125}I-labeled morphine (10,000 cpm) or [^{3}H]morphine (2500 cpm) solution. Equilibrium was reached within 4 hr at 4°C, but overnight incubations were also used. Assays were terminated by adding 100 μl of horse serum as carrier and precipitating with polyethylene glycol [M_r 8000, 12.5% (wt/vol) final concentration]. After centrifugation (5000 × g, 15 min, 4°C) the supernatant was discarded, and radioactivity of the pellet was determined. With [^{3}H]morphine the assay was performed directly in a 7-ml polyethylene vial; after aspiration of supernatant, 6 ml of CytoScint (WestChem Products, San Diego, CA) was added. Antiserum 937 was used at 3×10^{-7} final dilution with ^{125}I-labeled morphine as tracer, and antiserum S17 was used at 3×10^{-5} final dilution with [^{3}H]morphine as tracer. All determinations were in triplicate. IC_{50} values (concentrations for reduction of specific binding by 50%) were estimated by interpolation on a plot of B/B_0 against log concentration, where B and B_0 are tracer binding in presence and absence of test compound, respectively. A "total displacement control" (TD) was routinely determined, using 33 μM morphine (final concentration). Then the fraction of control specific binding was computed as $(B - TD)/(B_0 - TD)$. Occasionally (e.g., when screening column fractions or extracts), we

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Abbreviation: ir, immunoreactive.

extrapolated single-point data to estimate the IC_{50}. Limits of valid measurement in the RIA are considered to be 15–85%; values outside this range are expressed as less or greater than the amounts corresponding to these limits.

Partial Purification Procedure. For the experiment shown in Fig. 1 (*Upper*), 25 frozen beef hypothalami (400 g total, Pel-Freez) were homogenized in 5 volumes of 0.1 M acetic acid in a Waring blender, then simmered at 100°C for 30 min. After cooling, the extracts were filtered through glass wool and refrigerated overnight, then filtered through Whatman no. 1 paper on a Buchner funnel. The volume was reduced to about 350 ml on a rotary evaporator (bath at 45°C) after adding 0.05 vol of 1-butanol. The concentrated extract was adjusted to pH 9.0 with NH_4OH, and the resulting precipitate was removed by centrifugation (5000 × *g*, 10 min, 23°C). The supernatant was equilibrated by shaking in a separatory funnel with two successive equal volumes of chloroform containing 10% (vol/vol) 1-butanol. The partition coefficients (organic/aqueous) of morphine and normorphine in this system were determined to be 4.6 and 0.28, respectively. Pooled organic phases were back-extracted twice with equal volumes of 0.1 M HCl. The volume of the combined acid phases was reduced on the rotary evaporator to about 35 ml, pH was adjusted again to 9.0, and the entire phase-partition procedure was repeated, using one-tenth the original volume. The final HCl solutions were pooled, traces of organic solvents were removed on the rotary evaporator, and the material was lyophilized. The dried residue was redissolved in 800 μl of water and centrifuged 2 min in an Eppendorf Microfuge at 23°C, then 400 μl was injected onto a Waters C_{18} μBondapak column (3.9 mm × 30 cm) and was eluted with a 30-min linear gradient of 5–30% acetonitrile in 5 mM CF_3COOH (1.5 ml/min, 0.6-ml fractions). Absorbance was monitored at 228 and 280 nm. We call this reversed-phase system "HPLC-C." From each fraction, up to 10 μl was taken for RIA; when required, a 10-fold concentration was achieved by lyophilizing larger volumes in tubes to which RIA components were later added.

For the experiment shown in Fig. 1 (*Lower*), 20 frozen beef adrenal glands (290 g total) were extracted as described above, except that no volume reduction was carried out prior to the phase-partition procedure, and that phases were allowed to separate by standing overnight at 23°C. The final HCl back-extract was adjusted to pH 9.0 for adsorption chromatography. Amberlite XAD-2 beads (Mallinckrodt, 15 g) were washed twice with 100 ml of methanol, then three times with 100 ml of 0.1 M ammonium acetate buffer (pH 9.0), and poured into a 20 × 1 cm column. The extract was run onto the column (1 ml/min) at 23°C, followed by 50 ml of the buffer and then 50 ml of methanol. The methanol eluate was dried on the rotary evaporator and the residue was resuspended in 2 ml of 0.1 M HCl. Of this material, 50 μl was analyzed by HPLC-C, as described above.

Isolation Procedure. The same batch of frozen adrenals, which was exceptionally rich in ir-morphine (33 nmol/g by RIA with antiserum 937, 4.5 nmol/g by RIA with antiserum S17), was used. Extraction and preliminary purification by phase partition and XAD-2 chromatography were as described above. The following additional steps were employed, in the order given. (*a*) Cation-exchange chromatography on CM-Sephadex C-25 (Pharmacia): 20 × 1 cm column, elution at 0.5 ml/min with a linear gradient of NaCl to 0.5 M in 10 mM phosphate buffer (pH 7.0). Before samples were applied to ion-exchange columns, they were diluted to the same conductivity as the starting column buffer. Fractions containing ir-morphine were desalted as required on XAD-2, eluted with methanol, and dried on the rotary evaporator. (*b*) Anion-exchange chromatography on QAE-Sephadex A-25 (Pharmacia): 20 × 1 cm column, elution at 0.5 ml/min with a linear gradient of NaCl to 0.4 M in 1 M NH_4OH. (*c*) HPLC-M: Conditions as for HPLC-C, except elution was

Table 1. Crossreactivities in the morphine radioimmunoassays

Compound (Relationship to morphine)	% of morphine immunoreactivity (±SEM) with Antiserum 937	Antiserum S17	Ratio, 937/S17
Morphine	100.0 (0.36 ± 0.02)*	100.0 (0.91 ± 0.06)*	1.0
Dihydromorphine (7,8-dihydro)	29 ± 3	47 ± 4	0.62
Hydromorphone (6-keto 7,8-dihydro)	16 ± 0.5	0.46 ± 0.02	35
Levorphanol (7,8-dihydro, no O bridge or 6-OH)	7.8 ± 0.3	0.00096 ± 0.00023	8100
Dextrorphan (+-enantiomer of levorphanol)	0.0047 ± 0.0005	0.00063 ± 0.00007	7.5
Morphine 3-glucuronide	5.1 ± 0.2	0.014 ± 0.001	360
Codeine (3-methyl ether)	240 ± 9	0.083 ± 0.053	2900
Codeinone (3-methyl ether, 6-keto)	60 ± 3	0.029 ± 0.002	2100
Oripavine (6-methyl ether, $\Delta^{6-7, 8-14}$)	2.1 ± 0.1	0.048 ± 0.021	44
Thebaine (oripavine 3-methyl ether)	28 ± 3	0.00017 ± 0.00001	160,000
Normorphine (*N*-methyl removed)	0.27 ± 0.002	37 ± 6	0.0073
Oxymorphone (14-OH-hydromorphone)	0.045 ± 0.005	0.038 ± 0.008	1.2
Naloxone (*N*-allylnoroxymorphone)	0.095 ± 0.007	0.046 ± 0.007	2.1
Naltrexone (*N*-cyclopropylmethyl-noroxymorphone)	0.0068 ± 0.0008	0.068 ± 0.005	0.10
Tetrahydropapaveroline†	<0.0028	<0.0028	
Reticuline†	<0.0028	<0.0028	
Salsolinol†	<0.0028	<0.0028	
Sufentanil†	<0.0028	<0.0028	
Opioid peptides†	<0.0028	<0.0028	

Crossreactivities represent comparisons with morphine IC_{50} determined in the same experiment. Each estimate is based on three or four independent experiments done on different days.

*IC_{50} (nM), based on eight or more experiments with each antiserum.

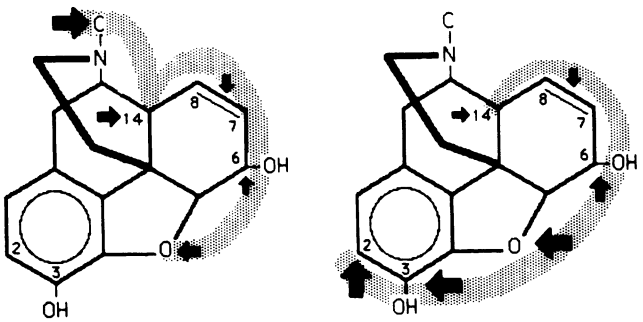
†None of these compounds inhibited binding at 3.3 μM final concentration, the highest concentration tested, from which a conservative maximum estimate of the crossreactivity was computed, as given here. Included in the group of inactive peptides were α-neoendorphin, β-neoendorphin, dynorphin A, dynorphin A(1–8), dynorphin B (rimorphin), [Met]enkephalin, [Leu]enkephalin, [Met]enkephalin-Arg-Phe, [Met]enkephalin-Arg-Gly-Leu, peptide E, peptide F, β_h-endorphin, β-casomorphin, morphiceptin, metorphamide, [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAGO), and dermorphin. Dynorphin B-29 (leumorphin) was not available for testing at >0.37 μM but showed no inhibition at that concentration. In these experiments, [^{3}H]morphine was used as tracer for the antiserum-937 RIA, with a final antiserum dilution of 3×10^{-6}.

with a gradient of 30–55% methanol in 0.03 M NH_4OH. (*d*) HPLC-C: As described above.

Preparation of Immunoaffinity Resin. Antiserum 937 and control normal sheep serum were precipitated with ammonium sulfate at 50% saturation. The washed and dialyzed immunoglobulin preparation (20 mg) was coupled to 5 g of cyanogen bromide-activated Sepharose-4B (Pharmacia) according to the manufacturer's instructions. More than 95% of the protein, as measured by absorbance at 280 nm, was coupled to the resin. The capacity of the packed hydrated 937 resin for morphine was 5 nmol per ml.

RESULTS

The specificity profiles of antisera 937 and S17 are shown in Table 1. Shading and arrows in the illustration below summarize data from the table, showing positions on the morphine molecule at which changes interfere with recognition by each antiserum. The larger the arrow, the greater the effect.



The antiserum S17 is intolerant of structural changes near position 3 of the morphine molecule, so it binds ^{125}I-labeled morphine very poorly; therefore only [^{3}H]morphine tracer was usually employed. With antiserum 937, results obtained with [^{3}H]morphine were substantially the same as with ^{125}I-labeled morphine, so the more convenient radioiodinated tracer was used. The recognition patterns for the various congeners of morphine reflect the different modes of conjugation in the immunogens. For example, codeine reacts strongly with 937 (immunogen conjugated through 3-OH) but weakly with S17 (conjugated through N), whereas normorphine does the opposite. The antisera recognize even minor changes such as saturation at the 7,8 double bond, and S17 shows a two-orders-of-magnitude decrease in affinity when 6-keto (in hydromorphone) is substituted for 6-OH (in dihydromorphine). The planar morphine precursors tetrahydropapaveroline and reticuline (15) do not crossreact with either antiserum. The strongly μ receptor-selective and potent synthetic opioid sufentanil is not recognized. Moreover, none of the opioid peptides crossreact at the highest concentrations that could be tested—not even the highly μ-selective enkephalin derivative DAGO (16). These findings confirm previous observations of others (17) that opioid peptides do not react with morphine antisera.

Taken as a whole, the antiserum specificities suggest strongly that any compound showing immunoreactivity with antiserum 937 or S17 must closely resemble morphine. The six immunoreactive peaks from both hypothalamus and adrenal (Fig. 1) therefore represent a family of compounds structurally related to morphine. To show that hypothalamus and adrenal contained the same six peaks, immunoreactive fractions constituting each peak were pooled, lyophilized, and dissolved in water. Corresponding numbered peaks from hypothalamus and adrenal, mixed peak for peak (equal amounts of immunoreactivity), were coeluted as single symmetrical peaks of immunoreactivity on HPLC-C.

The immunoreactivity ratios (937/S17) for peaks 1–6 from adrenal were, respectively, 1.8, >1500, >1000, 45, 97, and 520. Based on its immunoreactivity ratio near unity, the probable identity of peak 1 is morphine. Peak 1 was eluted at the same position as morphine, and furthermore, a mixture of immunoreactive equivalents of peak 1 and authentic morphine was coeluted as a single symmetrical peak of immunoreactivity. Further evidence that peak 1 is morphine is given below.

In general, the same pattern of immunoreactive peaks was observed in preparations from fresh and frozen tissues and in animals from ranches and feed lots. On the other hand, extremely large variations were found in the total amounts of immunoreactive material. For example, in one batch of 50 frozen hypothalami, the total ir-morphine (by antiserum-937 RIA) varied from undetectable (<0.25 pmol/g) in one-third of the samples to as high as 4.9 pmol/g, and even higher values have been found occasionally in other batches. High animal-to-animal variability was seen even in fresh slaughterhouse material from heifers raised in the same feed lot. Furthermore, analysis of coronal sections of fresh material revealed that adjacent regions of a single brain could differ by as much

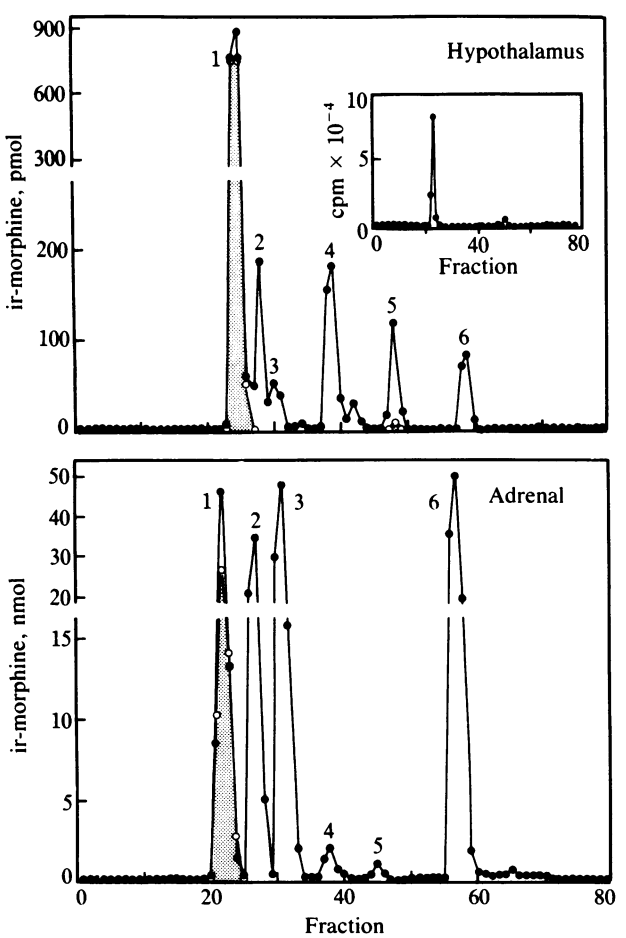


FIG. 1. HPLC elution profile of immunoreactive substances from beef hypothalamus (*Upper*) and adrenal (*Lower*). See the partial purification procedure under *Materials and Methods* for details of the preparations. Solid circles, ir-morphine by antiserum-937 RIA; open circles (shaded peaks), ir-morphine by antiserum-S17 RIA. (*Inset, Upper*) A separate experiment in which [^{3}H]morphine (ring-labeled, 0.25 μCi; 1 Ci = 37 GBq) was added to the hot acetic acid used to extract the hypothalami. Radioactivity under the single peak, which was eluted precisely at the morphine position, accounted for 54% of that added initially.

as a factor of 1000 in ir-morphine concentration. Sometimes we have found an exception to the rule of qualitative similarity, in that peak 1 (morphine) could be absent and another major peak appear instead, corresponding—in elution position, preferential recognition by antiserum S17, and bioactivity—to normorphine.

Several kinds of control experiments were done. The entire procedure was carried out on several occasions in the absence of tissue (i.e., blank runs), and no immunoreactivity was found. Fig. 1 also shows (*Inset, Upper*) that when 14 pmol of [^{3}H]morphine (ring-labeled) was added to the hot acetic acid used for extracting the tissue, it remained unchanged through the purification procedure and was recovered with 54% overall yield at the position of authentic morphine. The amount of added morphine was <5% of that already present. No significant amount of radioactivity was eluted anywhere else. Furthermore, when 50 nmol of normorphine was added to hot acetic acid in the absence of tissue, only a single peak of immunoreactivity was obtained on HPLC-C, which was coeluted with normorphine and had the appropriate immunoreactivity ratio. These controls rule out the possibility that the procedure itself converts morphine or normorphine to any other peak. When morphine 3-glucuronide was added at the outset in 100 times the amount of peak 1 (morphine), there was no increase in the amount of ir-morphine; thus, a glucuronide metabolite is not the source of the morphine obtained from tissues. Nevertheless, some of the immunoreactive compounds may have originated *de novo* during the workup by chemical modification of others. For example, we have found that peak 6 is unstable in the presence of NH_4OH, giving rise to peaks 2 and 3 under the alkaline conditions employed at the phase-partition step, during application to the XAD-2 column, and during anion-exchange chromatography on QAE-Sephadex.

Bioactivity was tested in the guinea-pig ileum preparation as described (18). The 50% inhibitory concentration of material in peak 1 in nine strips was found to be 180 ± 27 nM (concentration expressed in ir-morphine equivalents by antiserum 937 RIA). This value was in the range found for authentic morphine in our laboratory. The inhibition was promptly and substantially reversed by 10 nM naloxone. Peaks 2–6 were inactive at 4200, 1900, 26, 25, and 1200 nM, respectively—the highest concentrations available for testing.

In the full isolation procedure described under *Materials and Methods*, peak 1 from adrenal had been eluted in the same positions as morphine at every step—at 0.23 M NaCl on the cation exchanger, at 0.15 M NaCl on the anion exchanger, at fraction 43 on HPLC-M, and at fraction 23 on HPLC-C. Fig. 2 shows the analytical evidence for the purity of this material. Control resin, coupled to normal sheep globulins (Fig. 2 *A* and *B*) failed to remove either UV absorbance or immunoreactivity. Affinity resin, coupled to antibodies from serum 937, removed virtually all UV absorbance and immunoreactivity (*C*), which were then recovered by elution from the resin (*D*). The same material that was sampled for this experiment was also analyzed by NMR (see below). When it was mixed with an immunoreactive equivalent of partially purified adrenal peak 1 (see Fig. 1) and analyzed by HPLC-C, a single symmetrical peak of immunoreactivity was obtained at the position of authentic morphine.

Proton NMR spectra were obtained at 500 MHz for 1 μmol of morphine and ≈0.1 μmol of ir-morphine from peak 1. Exchange of labile protons and water in the samples was achieved by evaporation from 99.96% ^2H_2O under aspirator vacuum from a 45°C bath, repeated several times. The samples were transferred under N_2, dissolved in 100.0% [$^2H_5$]pyridine, and sealed for immediate use. Comparison of the spectra of peak 1 and morphine (Table 2) yielded excellent correspondence with respect to line shapes for all resonances. The differences in chemical shifts between the two

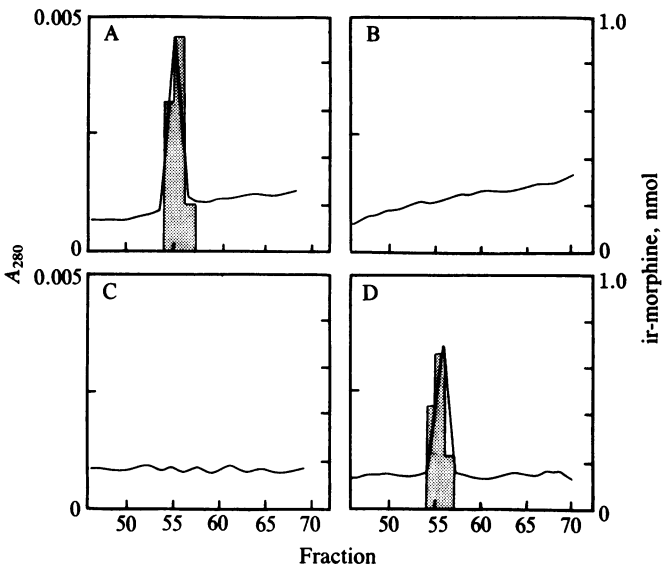


FIG. 2. Demonstration of purity of peak 1 by immunoaffinity resins. Hydrated 937-conjugated resin and control resin (1 ml each) were incubated (18 hr, 4°C) with 2 nmol ir-equivalent of peak 1 in 10 ml of 150 mM sodium phosphate buffer (pH 7.4) and then separated by filtration, and the filtrate was lyophilized. The resin was washed with buffer, incubated 2 hr at 23°C in 10 ml of 1 M acetic acid, and washed with an additional 10 ml of acetic acid. The pooled acetic acid eluates were lyophilized, dissolved in 1 ml of water, and analyzed on HPLC-C as described in *Materials and Methods*, except that a shallower gradient (0%–10%) was used. The detection limit in the 937 RIA was 8 pmol per fraction. (*A*) Filtrate, control resin. (*B*) Eluate, control resin. (*C*) Filtrate, affinity resin. (*D*) Eluate, affinity resin.

spectra varied from −0.01 to +0.07 ppm. Under the conditions required to obtain a spectrum of the extremely small sample of peak 1, a distorted baseline was obtained in the range δ 2.0–3.5; the minor discrepancies in chemical shifts were confined to this region. We conclude that peak 1 is morphine.

DISCUSSION

We have detected six peaks of ir-morphine in extracts of bovine hypothalamus and adrenal. Immunoreactivity ratio, adsorption to an anion exchanger at alkaline pH, coelution

Table 2. Proton NMR comparison of peak 1 to authentic morphine

Assignment	Chemical shift (δ)*		Coupling constant
	Peak 1	Morphine	*J*, Hz
H-2	7.04	7.03	8 (d)
H-1	6.68	6.68	8 (d)
H-8	6.05	6.04	10 (d,m)
H-7	—†	5.04	10 (d,m)
H-5	—†	—†	—
H-6	4.54	4.55	(m)
H-9	3.32	3.25	3, 6 (dd)
H-10β	3.03	3.00	18 (d)
H-14	2.85	2.79	(m)
H-16 ax.,eq.	2.4–2.5	2.4–2.5	(m)
N-CH_3	2.35	2.30	(s)
H-10α	2.34	2.30	6, 18 (dd)
H-15 ax.	2.08	2.04	(m)
H-15 eq.	1.75	1.74	12 (d,m)

Abbreviations: ax., axial; eq., equatorial; s, singlet; d, doublet; m, multiplet.
*Chemical shifts were calibrated against the upfield pyridine resonance at δ 7.19; spectra were measured in pyridine.
†Obscured by the residual $^1HO^2H$ resonance.

with authentic morphine in two HPLC systems, and bioassay potency and naloxone reversibility indicated that one peak (peak 1) was morphine. After purification to homogeneity, the structure was confirmed by NMR.

The specificities of the two antisera (Table 1) imply that any immunoreactive compound must closely resemble morphine. We conclude, therefore, that the remaining five peaks are very much like morphine; perhaps they are biosynthetic intermediates. All the peaks from hypothalamus were also found in adrenal, but in different relative proportions. A predominant peak from both tissues was peak 1 (morphine), and to the highest concentrations available for testing, this was the only bioactive one. The remaining peaks have ratios of immunoreactivities with the two antisera that suggest the presence of a substituent (such as methyl) on the 3-OH group; and a free 3-OH in the morphinans is essential for potency in bioassays. However, none of the unidentified peaks appears to be codeine, codeinone, oripavine, or thebaine, as judged by antiserum immunoreactivity ratios and coelution experiments on HPLC. By use of procedures like those described, peaks 2, 3, and 6 have been purified to homogeneity, but their structures are not yet known.

There is no doubt, then, that compounds closely related to morphine are present in bovine brain and adrenal. The multiplicity of substances effectively rules out laboratory contamination as a source—a possibility that would be difficult to exclude if only morphine had been found. Moreover, blank runs have been consistently negative. We have shown that none of the other peaks arises from morphine or normorphine during the course of our procedure and that the morphine itself is not generated from its glucuronide metabolite in the course of the purification. However, we cannot rule out the possibility that some opiate in tissue is converted to morphine after extraction. It is also evident that normorphine could be detected only if it were present in large amounts in tissue, in view of its unfavorable partition coefficient (organic/aqueous = 0.28, *cf.* 4.6 for morphine) at pH 9.0.

The major question is whether these opiates are endogenous or exogenous. The unusually high variability in the content of ir-morphine in different batches of hypothalamus and adrenal and in adjacent brain sections is compatible with either hypothesis. Only a demonstration of biosynthesis can settle the question decisively.

Note Added in Proof. Oka *et al.* (19) recently reported the purification of a substance from toad skin that appeared to be morphine by numerous criteria.

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