Pharmacology Biochemistry & Behavior, Vol. 23, pp. 295–301, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Pharmacokinetics of Morphine in Striatum and Nucleus Accumbens: Relationship to Pharmacological Actions

M. MELZACKA,* T. NEßELHUT,† U. HAVEMANN,[1]†
J. VETULANI* AND K. KUSCHINSKY‡

*Institute of Pharmacology, Polish Academy of Sciences, PL-31-343 Kraków, Poland
†Department of Biochemical Pharmacology, Max Planck Institute for Experimental Medicine
D-3400 Göttingen, F.R.G.
‡Institute of Pharmacology and Toxicology, School of Pharmacy
University of Marburg, D-3550 Marburg, F.R.G.

Received 14 September 1984

MELZACKA, M., T. NEßELHUT, U. HAVEMANN, J. VETULANI AND K. KUSCHINSKY. *Pharmacokinetics of morphine in striatum and nucleus accumbens: Relationship to pharmacological actions.* PHARMACOL BIOCHEM BEHAV **23**(2) 295–301, 1985.—The pharmacokinetics of morphine was compared with its ability to increase striatal dopamine turnover (estimated by an increase in DOPAC concentration) and to produce the development of a muscular rigidity (estimated as a tonic activity in the electromyogram). After systemic administration of morphine (15 mg/kg IP), the concentration of morphine in blood plasma, striatum and substantia nigra showed a parallel time course with a maximum after 30 min; in the striatum, in addition, normorphine was found in a lower concentration, but with a similar time course. The elevation of striatal DOPAC, in contrast, commenced very rapidly and lasted for about four hours. The rigidity appeared later and disappeared earlier than the striatal DOPAC elevation. After unilateral intrastriatal injection of morphine (15 μg), a small amount of the drug penetrated very rapidly to distant sites, such as the contralateral striatum and nucleus accumbens, as well as to the ipsilateral nucleus accumbens. The results suggest that the relationship between pharmacokinetics and pharmacodynamics of morphine, both after systemic and after local injection into the brain, is more complex than could be expected from previous findings.

Morphine pharmacokinetics Striatal dopamine turnover Muscular rigidity

IN many cases, attempts to correlate pharmacokinetic data of morphine with a pharmacological effect have been limited to studies about the relationship between cerebral concentration of morphine and its analgesic action, and the correlations found were poor [3,14]. This is not surprising, since an important part of morphine-induced analgesia is due to its action on the spinal cord [19], and in fact, in recent studies, it was found that there is a good correlation between morphine-induced analgesia and the drug's concentration in the spinal cord [1].

Another effect of morphine, which is most pronounced in the rat and is due to supraspinal actions, is its effect on motor functions, namely akinesia and its more pronounced form, catalepsy, on one hand and muscular rigidity on the other; the co-existence of both signs is termed 'catatonia.' In previous studies, it was shown that the rigidity results from an interaction of morphine with opioid receptors in the striatum, whereas catalepsy is most probably due to an action of morphine in the nucleus accumbens (for reference see [8]). Furthermore, it was shown that morphine increases the dopamine turnover in the striatum, which effect is at least in part mediated by opioid receptors in the substantia nigra, perhaps with an additional involvement of opioid receptors located on dopaminergic terminals in the striatum [8]. In the present study it was tested if there is any correlation between the concentration of morphine (and its most important metabolite, normorphine) in various parts of the basal ganglia and the degree of muscular rigidity and increase in dopamine turnover in the striatum after systemic administration of morphine. Moreover, since unilateral injection of morphine into the striatum produces muscular rigidity as well [9], we investigated the concentration of morphine in striatum and nucleus accumbens on both sides after unilateral intrastriatal injection of morphine (in order to obtain data on the spread of morphine after injection), and the resulting muscular rigidity was tested.

METHOD

Male albino Wistar rats (TNO/W 70 of F. Winkelmann, Borchen, F.R.G.) of 220–270 g were used. Morphine hydrochloride (E. Merck, Darmstadt, F.R.G.) was dissolved in saline and administered either intraperitoneally (IP) or into

[1]Requests for reprints should be addressed to U. Havemann, Max Planck Institute for Experimental Medicine, Department of Biochemical Pharmacology, Hermann-Rein-Str. 3, D-3400 Göttingen, F.R.G.

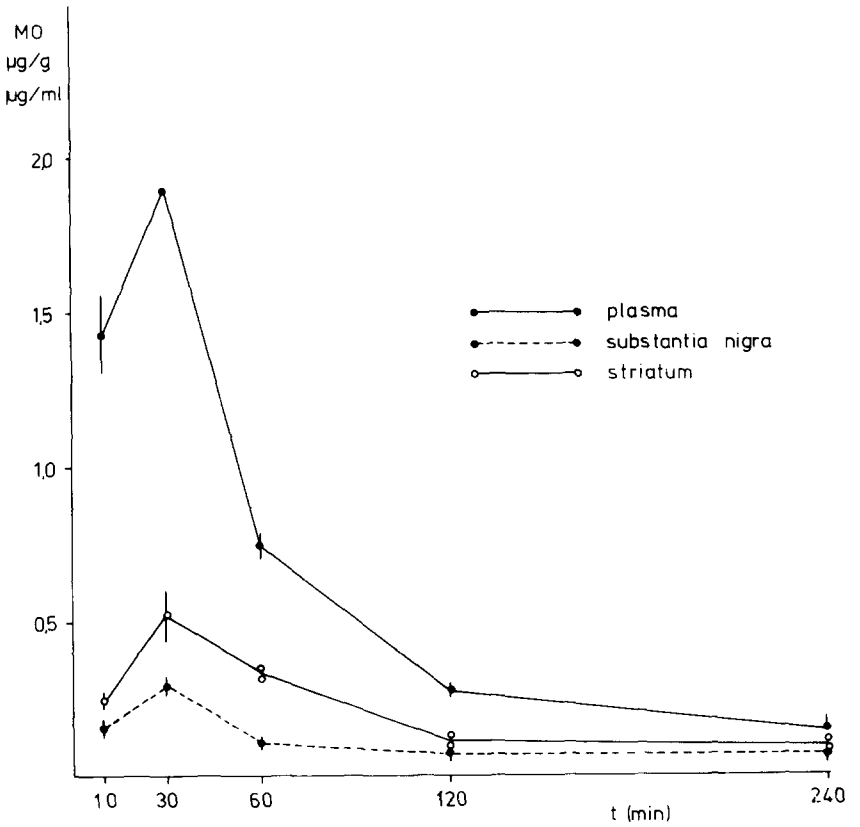

FIG. 1. Time courses of the concentration of morphine (MO) in blood plasma, substantia nigra and striatum, measured in an extract with perchloric acid ('acid extract') after administration of 15 mg/kg morphine IP. Means of 2–3 values±S.E. Abscissa: time after morphine injection (min), ordinate: concentration of morphine in plasma (µg/ml) and in substantia nigra and striatum (µg/g tissue).

the left striatum. The doses were expressed as the free base. At predetermined time-intervals after morphine administration, the animals were killed by cervical fracture and subsequent decapitation, the brain taken out and the striatum, and in some experiments also the nucleus accumbens and the substantia nigra of both sides, were isolated; furthermore, blood plasma was obtained from some of the animals.

Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), morphine and normorphine were estimated by using high performance liquid chromatography (HPLC).

Estimation of Dopamine and DOPAC in an 'Acid Extract'

Both catecholamines were extracted after homogenization of the brain in 200–500 µl of 0.4 M perchloric acid and centrifugation at 20,000 g for 30 min at about 4°C. The supernatant was analysed by using HPLC. The chromatograph (BCMA JN1, ERC GmbH, Alteglofsheim, F.R.G.) was equipped with a Nucleosil 3C-18 (Machery-Nagel, Düren, F.R.G.) reversed-phase column and an electrochemical detector with glassy carbon working electrode and an Ag/AgCl reference electrode. The detector was adjusted to 0.5 nA with an oxidation potential of 0.8 V. The mobile phase consisted of 0.02 M sodium citrate buffer, pH=4.2, containing 0.2 mM octane-1-sulfonic acid and 10% methanol.

Estimation of Morphine and Normorphine in a 'Neutral Extract'

The extraction was performed according to Kupferberg *et al.* [11]. Brain tissue was homogenized in 500 µl of saline solution at about 4°C. To the whole homogenate or to 1 ml of plasma, 0.2 g of solid Na_2CO_3 was added and 1 ml of a 10% solution of n-butanol in chloroform. This mixture was shaken mechanically for 20 min and centrifuged for 15 min at 12,000 g. The (upper) inorganic phase was removed by suction and 500 µl of the remaining organic phase was transferred into another tube containing 500 µl of 0.01 N HCl. It was shaken mechanically for 10 min, centrifuged for 10 min at 12,000 g, and 10–25 µl of the upper, acid phase was analysed by using HPLC as described above. The mean recoveries of morphine were 86.5% and that of normorphine 73.3%.

For *intrastriatal injections* of morphine the skull was fixed in a David Kopf stereotaxic apparatus under pentobarbital anesthesia and a permanent guide cannula was implanted unilaterally (above the left striatum), the tip being 4 mm above the injection site desired. After 3–5 days, the drug was slowly injected (<0.25 µl/min, total volume 1.5 µl) through a fine cannula (outer diameter 0.4 mm) into the head of the caudate nucleus, applying the coordinates given by

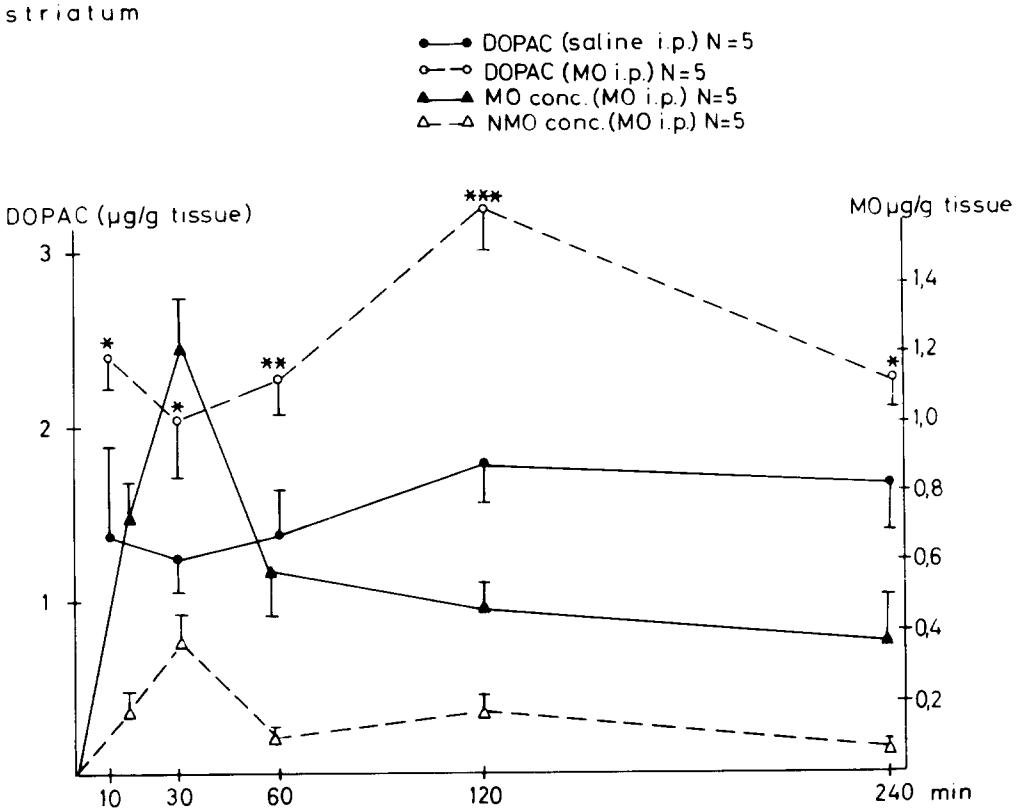

FIG. 2. Time courses of striatal DOPAC concentration (estimated in an 'acid extract') after administration of saline or of morphine (15 mg/kg IP) and of the striatal concentration of morphine (MO) and normorphine (NMO), both estimated in 'neutral extract' after injection of morphine (15 mg/kg IP). Abscissa: time (min) after morphine injection, ordinate left scale: striatal DOPAC concentration (µg/g tissue), ordinate right scale: striatal morphine concentration (µg/g tissue). Mean values±S.E., significances: *$\alpha<0.05$; **$\alpha<0.01$; ***$\alpha<0.001$ for DOPAC concentration of morphine-treated rats vs. saline-treated rats at the same time (Student's t-test).

Fifková and Maršala [4]: AP: −1.25; L: 2.6; V: 4.5. We selected this part of the caudate nucleus, because there is a higher density of opioid-specific binding sites than in the body or the tail of this nucleus [2,6]. Local injections of morphine into the striatum were performed slowly, in a manner reducing its spreading to other brain areas as much as possible [10,16].

The *tonic activity in the EMG* was recorded from the gastrocnemius-soleus (GS) muscle of the hindlimb of unanaesthetized rats. The method used is described elsewhere [9]. The animals were put individually into ventilated polyacryl boxes and their hind-limbs hung through slots in the bottom of the box. The hind-limb studied (the left) was gently fixed with adhesive tape. The activity in the EMG was recorded with pairs of percutaneously inserted, teflon-insulated stainless-steel fine wire electrodes (Cooner Wire, AS 632 SS) sampling EMG changes from the muscle under investigation. The signal was amplified, band-pass filtered (5 Hz-to 10 kHz), rectified and fed into an integrator, which automatically reset after a predetermined voltage was reached. The reciprocal of the rest-time of the integrator was the measure of the activity in the EMG. The EMG was recorded continuously and the values of the mean activity for 5 min periods were calculated. In general, the values of the activity recorded in the EMG were expressed as the quotient of the values recorded of the largest activity found in all experiments of this study.

RESULTS

In the first series of experiments, 'acid extracts' were used for estimation of dopamine and DOPAC as well as of morphine and normorphine in the same sample. The recoveries of dopamine and DOPAC were sufficiently high (86% and 89%) and, therefore, this method was used for the estimation of both substances. The recoveries of morphine, however, were less than 40% and normorphine was not detectable. Therefore, in the subsequent experiments, 'neutral extracts' were applied for estimation of both these drugs.

Figure 1 shows the time course of changes in morphine concentration (measured in acid extract), after its intraperitoneal injection, in the blood plasma and two regions of the brain: the striatum and substantia nigra. In all tissues the drug concentration increased rapidly within the first 10 min, and reached the peak approximately after 30 min, and then declined during the following 30 min. After that time the morphine level in the substantia nigra stabilized at approximately 30% of the maximum concentration for at least 3 hr; the course of changes in the striatum was similar, although some decline was also observed between 60 and 120 min

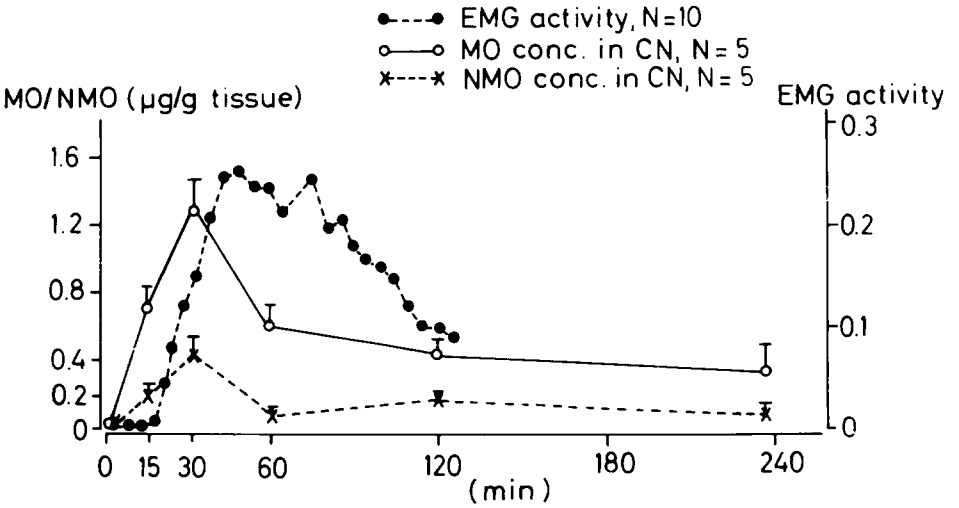

FIG. 3. Time courses of morphine (MO) and of normorphine (NMO) concentrations, estimated in 'neutral extract' in the striatum (CN) and of the tonic EMG activity after injection of morphine (15 mg/kg IP). Abscissa: time (min) after morphine injection, ordinate left scale: striatal morphine concentration (μg/g tissue), ordinate right scale: EMG activity. Morphine concentrations are indicated as mean values±S.E., EMG activities as median values.

after morphine administration. In the blood plasma the drug level continued to decline till 120 min after the injection and then stabilized at approximately 10% of the peak value.

A more detailed study was performed in the striatum, and all subsequent estimations of morphine and normorphine were performed by using 'neutral extracts.' In these studies (Fig. 2), it was found that normorphine was present together with morphine in this region: the changes in its concentration paralleled those in the concentration of the parent compound, but the tissue concentration of normorphine was only about 20% of the morphine concentration. The time course of the first phase of decline of both substances was roughly estimated to be 0.5 hr, whereas the subsequent phase showed a very slow decline.

The striatal concentration of DOPAC was significantly increased as early as 10 min after morphine administration, and then a second, slow increase seemed to appear, with a maximum after 120 min. The DOPAC level declined after that time, but even after 240 min, it had not fully reached the control value (Fig. 2). The striatal concentration of dopamine was not altered 120 min after administration of morphine (control value: 16.53±1.45 μg/g tissue±S.E., after morphine injection: 16.82±0.87 μg/g tissue±S.E.).

A comparison of the time course of striatal morphine concentration and the degree of muscular rigidity (Fig. 3) shows that the tonic EMG activity set in with some delay as compared with the increase in the concentration of morphine. The maximum of muscular rigidity was observed 45–60 min after injection of morphine, after 120 min, it had declined by about 70%. Thus the latency in decline was even more pronounced than that of onset.

In a few studies, the concentration of morphine was measured in the nucleus accumbens after systemic morphine administration. The results showed that the drug accumulated in this tissue more rapidly than in the striatum: the drug concentrations (μg/g tissue±S.E.M.) were 1.91±0.18 after 15 min and 2.17±0.21 after 30 min (N=3 for each value). No normorphine could be detected.

In experiments applying intrastriatal injection of morphine, it has been found that of the 15 μg injected, about one half (8 μg) were recovered from the injected (left) striatum 15 min after the injection (Table 1), about 4 μg after 30 min, and after 60 min, the value was slightly below 1 μg. In addition, a trace amount of normorphine was observed. In the contralateral (right) striatum, the total amount of morphine after 15 min was by two orders of magnitude lower (80 ng) as compared with the injected striatum, and the drug level declined to less than 20 ng after 30 min and to an almost undetectable level after 60 min. The total amount of morphine both in the ipsi- and contralateral nucleus accumbens was similar and only slightly lower than in the contralateral striatum after 15 min, indicating an almost threefold higher concentration in the tissue, since the nucleus accumbens has a lower weight than the striatum. The amount of morphine in the nucleus accumbens of both sides was very low after 30 min and close to the detection limit after 60 min. Semilogarithmic plot of the morphine concentrations in these four areas (not shown) suggested a monophasic decline in the injected striatum but a biphasic one in the non-injected striatum and in the nuclei accumbentes of both sides.

DISCUSSION

The muscular rigidity induced by systemic administration of morphine results from the drug interaction with striatal opioid receptors, whereas the morphine induced increase in the striatal dopamine turnover (reflected by elevated DOPAC concentration) is, at least in part, mediated by the opioid receptors located in the substantia nigra (though opioid receptors on the dopaminergic terminals in the striatum may possibly be also involved) [8]. Interestingly, the time courses of these phenomena after intraperitoneal

TABLE 1

TOTAL AMOUNT OF MORPHINE (MO) (ng), AFTER UNILATERAL INJECTION OF MORPHINE (15 μg) INTO THE LEFT STRIATUM, FOUND EITHER IN THE LEFT (INJECTED) STRIATUM (CN), OR IN THE RIGHT (NON-INJECTED) STRIATUM OR IN THE LEFT OR RIGHT NUCLEUS ACCUMBENS (ACB). MORPHINE WAS ESTIMATED IN A 'NEUTRAL EXTRACT' 15, 30 OR 60 MIN AFTER INTRASTRIATAL INJECTION. MEAN VALUES ± S.E.

Brain Area	time (min) post injection into the left CN 15	30	60
left CN (injected) N=5	8410 ± 795	3815 ± 480	850 ± 185
right CN N=5	79 ± 21	13 ± 1.8	3 ± 1
left ACB N=5	55 ± 15	10 ± 1.2	4.5 ± 1.6
right ACB N=5	60 ± 11	5 ± 1	1.5 ± 0.5

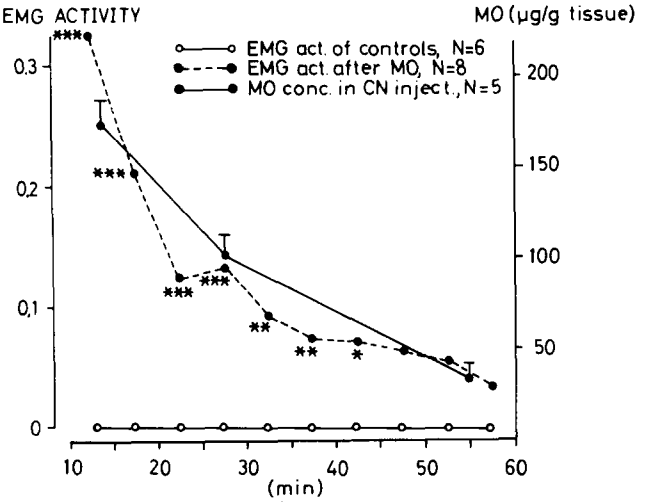

FIG. 4. The effect of morphine (MO) (15 μg) or of saline, injected into the left striatum, on the tonic activity in the EMG of the ipsilateral gastrocnemius-soleus (GS) muscle and the MO concentration in the striatum (CN) after intrastriatal injection (estimated in a 'neutral extract'). Left ordinate: EMG activity, median values, right ordinate: MO concentration (μg/g tissue), mean values±S.E., abscissa: min after intrastriatal injection. Significances: *α<0.05; **α<0.01; ***α<0.002 for EMG activity of morphine-treated rats vs. saline-treated rats at the same time (Mann-Whitney U-test).

administration of morphine are clearly different from the time course of morphine concentrations in those supposed target areas.

Striatal Dopamine Turnover

The increase in striatal DOPAC concentration reflecting an increase in dopamine turnover, was very rapid and preceded the rise in striatal and nigral concentrations of morphine. A particularly high affinity of morphine to opioid receptors may be responsible for such a rapid response. On the other hand, the decline of striatal DOPAC concentration occurred more slowly than the disappearance of morphine from the striatum and substantia nigra. It might be argued that under the experimental conditions the equilibrium between tissue concentration of morphine and receptor occupation had not been achieved, and that the observed decline in drug concentration was not reflecting the decrease in morphine-receptor interaction.

The time required for transformation of dopamine into DOPAC is relatively short and its clearance from the brain occurs very rapidly [18] resulting in an apparent $t^1/_2$ of about 10 min for DOPAC. Accordingly, this phenomenon cannot sufficiently explain the time-lag.

Obviously, the tissue concentrations of morphine may not necessarily reflect the degree of opiate receptor occupation. Only a minor fraction of morphine, present in micromolar concentrations in the brain, may be bound to opiate receptors, whose concentrations are lower by 2–3 orders of magnitude [15], and thus most of the drug must be either free, or unspecifically bound. The degree of receptor occupation, and therefore the pharmacological effect, may be related directly to the drug tissue concentration only under equilibrium conditions, and this requires very rapid equilibration process. The discrepancy between the time course of morphine effects and morphine concentration in target brain areas suggests that at least one of the transfer processes is relatively slow. Alternatively, the discrepancy might occur because of another action of the drug in another brain area, which modifies the effect generated from the region in which drug concentration is monitored.

Another possibility, that the discrepancy results from the combined action of the drug and its active metabolites, does not seem to be important in the present experiment, as the only active morphine metabolite, normorphine, has a similar potency to morphine [12] and its concentration paralleled closely that of morphine.

A likely explanation seems to be that the slow decline of morphine-induced increase in striatal dopamine turnover might be due to re-distribution phenomena of morphine between various local or supraspinal compartments, supplying opioid receptors being responsible for the increase of DOPAC for a certain time during morphine's clearance off the brain, so that the fractions of receptors occupied by morphine were higher than can be assumed from the surrounding tissue.

Muscular Rigidity

The time course of muscular rigidity was different both from the time course of the effect of morphine on striatal dopamine turnover and of morphine concentration in the striatum.

It developed rather slowly, so that its peak appeared later than the peaks of striatal DOPAC and morphine concentrations. The decline of rigidity appeared 45 min later than that of morphine concentration, although much earlier than the normalization of dopamine turnover in the striatum.

This peculiar time course of rigidity might be a result of interference of a dopaminergic activation with the rigidity. The activation of the dopaminergic neurones probably occurs via opioid receptors in the substantia nigra and is likely

to antagonize the rigidity [16], which in fact can be inhibited by dopaminergic drugs [17]. Furthermore, there is evidence that the receptors mediating the activation of dopaminergic neurones are slightly more sensitive to morphine than those mediating rigidity [7]. If these assumptions are valid, then the dopaminergic activation should induce a delay and an earlier termination of the rigidity, which in fact was suggested by the results.

Intrastriatal Injections

The decline of morphine from the striatum after slow intrastriatal injection was rapid: only approximately one half of the injected drug was present in the tissue after 15 min, and after 1 hr only 10% of the dose was present. There was a good time correlation between the changes in striatal morphine concentration and the degree of muscular rigidity, which appeared without a lag phase after intrastriatal morphine injection. This shows that in principle, a relatively rapid dissociation of morphine from its receptors in the striatum is possible, confirming many observations in in-vitro-preparations. Nevertheless, the rate-limiting step in the speed of decline of the rigidity might be the clearance off the tissue injected. If this is the case, the rate of dissociation of morphine from its receptors might even be underestimated.

From the rapid loss of morphine from the striatum injected, the question arises about the fate of the disappearing drug. Morphine appeared in minute quantities, but rather rapidly, in the contralateral striatum and both ipsi- and contralateral nuclei accumbentes. This observation and the fact that the concentrations both in the ipsilateral and contralateral nuclei accumbentes were similar suggests that the drug was not spreading only by passive diffusion through the tissue, although this might be relevant near the injection site, since morphine is a hydrophilic substance with a slow local diffusion rate [13]. A considerable portion of morphine was probably transported by convection via blood or extracellular fluid to distant sites.

Nucleus Accumbens

Accumulation of morphine in the nucleus accumbens proceeded more rapidly and up to a higher concentration level than in the striatum, the maximal concentration being observed already after 15 min. Since previous studies have suggested that opioid receptors in the nucleus accumbens play an important role in the development of akinesia and catalepsy [8], the present findings seem to be in good agreement with the particularly rapid onset of akinesia following systemic administration of morphine.

Conclusions

Relationship between the concentration of morphine in certain brain areas and its pharmacological effects is more complex than previously expected, particularly after systemic drug administration. The apparent discrepancies may be caused by slow equilibration processes as well as by mutually antagonistic, pharmacological effects of simultaneous stimulation of opioid receptors in various brain areas, with slight differences in receptor affinities. The importance of opioid receptors in a certain brain area for a specific pharmacological response may be evaluated by investigating the effect of local injection and the correlation between the local drug concentration and the resulting effect. However, it remains necessary to prove that either the drug does not penetrate into other brain areas in significant amounts during the experiment, or otherwise that the transfer of the drug to distant areas is not pharmacologically relevant under the experimental conditions and for the parameters studied. This underlines the importance of control injections into areas adjacent to the region studied, if possible in different directions in relation to the brain area studied.

ACKNOWLEDGEMENTS

This study was supported by a grant (B 10) of the SFB 33 'Nervensystem und biologische Information' of the Deutsche Forschungsgemeinschaft. The skillful technical assistance of Ch. Bode and H. Kügler is gratefully acknowledged.

REFERENCES

1. Adamus, A., M. Melzacka and J. Vetulani. Behavioral and pharmacokinetic interaction between morphine and haloperidol in the rat. *Pol J Pharmacol Pharm* **36:** 51–58, 1984.
2. Atweh, S. F. and M. Y. Kuhar. Autoradiographic localization of opiate receptors in rat brain. III. The telencephalon. *Brain Res* **134:** 393–405, 1977.
3. Dahlström, B. E. and L. K. Paalzow. Pharmacokinetics of morphine in plasma and discrete areas of rat brain. *J Pharmacokinet Biopharm* **3:** 293–302, 1975.
4. Fifková, E. and J. Maršala. Stereotaxic atlases for the cat, rabbit and rat. In: *Electrophysiological Methods in Biological Research*, edited by J. Bureš, M. Petrán and J. Zachar. New York: Academic Press, 1967, pp. 653–696.
5. Fishman, J., E. F. Hahn and B. J. Norton. N-demethylation of morphine in rat brain is localized in sites with high opiate receptor content. *Nature* **261:** 64–65, 1976.
6. Fonnum, F. and I. Walaas. Localisation of neurotransmitter candidates in neostriatum. In: *The Neostriatum*, edited by I. Divac and R. G. E. Öberg. Oxford: Pergamon Press, 1979, pp. 53–59.
7. Genç, E., U. Havemann, N. Tzoneva-Tyutyulkova and K. Kuschinsky. Motility, rigidity and turnover of dopamine in the striatum after administration of morphine to rats: A re-evaluation of their mechanisms. *Neuropharmacology* **22:** 471–476, 1983.
8. Havemann, U. and K. Kuschinsky. Neurochemical aspects of the opioid-induced 'catatonia.' *Neurochem Internat* **4:** 199–215, 1982.
9. Havemann, U., M. Winkler and K. Kuschinsky. Opioid receptors in the caudate nucleus can mediate EMG-recorded rigidity in rats. *Naunyn Schmiedebergs Arch Pharmacol* **313:** 139–144, 1980.
10. Jacquet, Y. F. and A. Lajitha. Morphine action at central nervous system sites in rat: analgesia or hyperalgesia depending on site and dose. *Science* **182:** 490–492, 1973.
11. Kupferberg, H., A. Burkhalter and E. L. Way. A sensitive fluorometric assay for morphine in plasma and brain. *J Pharmacol Exp Ther* **145:** 247–251, 1964.

12. Kosterlitz, H. W., J. A. H. Lord, S. J. Paterson and A. A. Waterfield. Effects of changes in the structure of enkephalins and of narcotic analgesic drugs on their interactions with μ- and α-receptors. *Br J Pharmacol* **68:** 333–342, 1980.
13. Lomax, P. The distribution of morphine following intracerebral microinjection. *Experientia* **22:** 249–250, 1966.
14. Plomp, G. J. J., R. A. A. Maes and J. M. van Ree. Disposition of morphine in rat brain: Relationship to biological activity. *J Pharmacol Exp Ther* **217:** 181–188, 1981.
15. Snyder, S. H. and J. P. Bennet. Neurotransmitter receptors in the brain: Biochemical identification. *Annu Rev Physiol* **38:** 153–175, 1976.
16. Turski, L., U. Havemann and K. Kuschinsky. Evidence for functional interactions of morphine in substantia nigra and striatum in relation to muscular rigidity in rats. *Neurosci Lett* **28:** 291–296, 1982.
17. Wand, P., K. Kuschinsky and K. H. Sontag. Morphine-induced muscular rigidity in rats. *Eur J Pharmacol* **24:** 189–193, 1973.
18. Westerink, B. H. C. and J. Korf. Turnover of acid dopamine metabolites in striatal and mesolimbic tissue of the rat brain. *Eur J Pharmacol* **37:** 249–255, 1976.
19. Yaksh, T. L. and T. A. Rudy. Narcotic analgetics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. *Pain* **4:** 299–359, 1978.