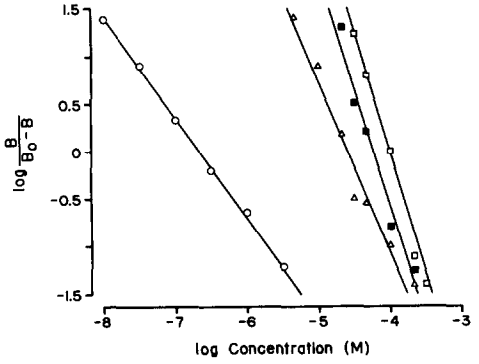

Fig. 2. Hill plots for displacements of [^{3}H]ouabain specific binding to guinea pig heart ($Na^+ + K^+$)ATPase by ouabain (○), arachidonic acid (△), oleic acid and (■) and linoleic acid (□). All displacing agents were assayed on three different ($Na^+ + K^+$)ATPase preparations; each point corresponds to the mean value. B_0 is the radioligand binding in the absence of displacing agent; B is the radioligand binding in the presence of displacing agent.

In summary, displacement of [^{3}H]ouabain specific binding to guinea pig heart ($Na^+ + K^+$)ATPase was produced by unlabeled ouabain, by a partially purified extract from guinea pig heart and by arachidonic, oleic and linoleic acids. Ouabain and the extract interacted with the radioligand at the binding site in a competitive manner, whereas fatty acids produced non-Michaelis displacements. Therefore, the extract activity is not likely due to the presence of fatty acids, but to an endogenous factor that binds to the digitalis binding site.

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Biochemical Pharmacology, Vol. 36, No. 10, pp. 1738–1741, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
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Effects of opioid agonist drugs on the *in vitro* release of ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT from slices of rat globus pallidus

(*Received* 3 *July* 1986, *accepted* 4 *December* 1986)

The strio-pallidal enkephalin-containing pathway appears to influence locomotor activity and circling behaviour [1, 2]. But how enkephalins alter neuronal activity in the globus pallidus has not been investigated. Enkephalins may act to modulate afferent input to pallidum, or may directly alter the activity of output neurones.

The rat globus pallidus receives projections from many brain regions including the striatum [3, 4], substantia nigra [5], subthalmic nucleus [6, 7], nucleus accumbens [8, 9] and dorsal raphe nuclei [10, 11]. The strio-pallidal projection contains γ-aminobutyric acid (GABA) and this projection is involved in the mediation of circling behaviour [12]. Similarly, pallidal afferents from the nucleus accumbens also contain GABA but influence locomotor activity [13]. Collaterals extending from the nigro-striatal pathway may provide dopaminergic innervation to the globus pallidus [15]. While fibres projecting from the dorsal raphe nucleus give rise to 5-hydroxytryptamine (5HT) containing terminals [11].

In this work we have examined the action of opioid agonist drugs on the release of GABA, dopamine and 5HT in the globus pallidus. We have examined the effects of opiate agonists previously shown to induce circling or locomotor response on intrapallidal injection [1] to alter the release of ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT from pallidal slices.

Materials and methods

Tissue preparation and prelabelling of pallidal slices with ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT. Pallidal tissue from individual female Wistar rats (151–175 g; Charles River Ltd) was chopped in two directions (0.2 mm × 0.2 mm) using a McIlwain tissue chopper (Mickle Engineering Co. Ltd.). The resulting pallidal slices were dispersed in 1.0 ml of oxygenated Krebs buffer, pH 7.4, at 37°. Slices were prelabelled with ^{3}H-GABA (74 Ci/mmol; Amersham International), ^{3}H-dopamine (13.6 Ci/mmol. Amersham International) or ^{3}H-5HT (21 Ci/mmol; Amersham

International), added to the incubates to give final concentrations of 1×10^{-7} M; 2.5×10^{-7} M and 6×10^{-7} M respectively, over a 15 min period at 37°. The prelabelled pallidal slices were superfused with Krebs buffer at 37°, constantly gassed with 95% O_2/5% CO_2, at a rate of between 0.8 and 1.0 ml/min for 30 min. At the end of 30 min the spontaneous release of radioactivity reached a constant level. At this time the perfusate was collected serially for 2 min periods (1 fraction) over the following 28 min (total 14 fractions).

The effect of opioid agonist drugs on the spontaneous release of ^{3}H-*GABA,* ^{3}H-*dopamine and* ^{3}H-*5HT.* Three fractions of perfusate from pallidal slices were collected prior to, and 11 fractions were collected after, the addition of ethylketocyclazocine methanesulphonate (50 and 100 μM; EKC Sterling Winthrop), D-Ala^{2}D-Leu^5-enkephalin; (50 and 100 μM; DADLE; BW 180C; Wellcome Laboratories) or Tyr-D-Ala-Gly-MePhe-Met(O)-ol (50 and 100 μM; FK 33–824; Sandoz) to the perfusing medium.

The effect of KCl on the release of ^{3}H-*GABA,* ^{3}H-*dopamine and* ^{3}H-*5HT.* Following the collection of three fractions to assess basal efflux of radioactivity, KCl (25 mM) was included in the perfusing Krebs buffer for a further period of two fractions. The tissue was then again perfused with Krebs buffer alone for a further nine fractions. The effect of removing calcium from the perfusing medium on the KCl-evoked release of ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT was investigated by replacing $CaCl_2.6H_2O$ osmotically with $MgCl_2.6H_2O$.

The effects of opioid agonist drugs on the KCl-*evoked release of* ^{3}H-*GABA,* ^{3}H-*dopamine and* ^{3}H-*5HT.* Following the collection of one fraction, EKC (50 and 100 μM), DADLE (50 and 100 μM) or FK 33-824 (50 and 100 μM) were included in the perfusing Krebs buffer for a period of four fractions. KCl (25 mM) was included in the perfusing Krebs buffer for the last two of these fractions; thus, opioid agonist drugs were present in the perfusing Krebs buffer for two fractions prior to and then during the two fractions of KCl stimulation. Following KCl addition the tissue was again perfused with Krebs buffer alone for a further nine fractions.

Statistical analysis. Differences between ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT release in the presence and absence of opioid agonists were analysed using a two-tailed Student's *t*-test.

Results

As an indication of the extent of uptake of ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT, the total amount of radioactivity calculated to be present in the pallidal tissue preparations prior to perfusion in four separate experiments was as follows: ^{3}H-GABA, 658,469 ± 45,592 cpm; ^{3}H-dopamine, 232,467 ± 15,051 cpm; ^{3}H-5HT, 387,082 ± 37,872 cpm.

The effects of opioid agonist drugs on the spontaneous release of radioactivity from pallidal slices. The spontaneous release of radioactivity from pallidal slices preincubated with ^{3}H-5HT, ^{3}H-dopamine or ^{3}H-GABA was unaffected by perfusion of the tissue preparation with EKC, DADLE and FK 33-824 (50 and 100 μM).

Calcium-dependent KCl-*evoked release of radioactivity from pallidal slices preincubated with* ^{3}H-*GABA,* ^{3}H-*dopamine or* ^{3}H-*5HT.* The inclusion of KCl (25 mM) in the perfusing Krebs buffer increased the rate of efflux of radioactivity from slices of globus pallidus preincubated with ^{3}H-GABA, ^{3}H-dopamine or ^{3}H-5HT. In all three cases KCl-evoked release of radioactivity was dependent on the presence of calcium. The replacement of calcium chloride with an equivalent concentration of magnesium chloride in the perfusing Krebs buffer abolished the increase in release of radioactivity stimulated by KCl.

The effect of opioid agonist drugs on the KCl-*evoked release of radioactivity from pallidal slices preincubated with* ^{3}H-*GABA,* ^{3}H-*dopamine or* ^{3}H-*5HT.* KCl-evoked release of radioactivity from pallidal slices pre-incubated with ^{3}H-GABA was reduced by exposure of the tissue (for 2 min prior to and then during exposure to KCl) to the δ-receptor agonist, DADLE (50 μM). However, a higher concentration of DADLE (100 μM) failed to have any effect on the KCl-evoked release of radioactivity (Fig. 2). The KCl-evoked release of radioactivity from pallidal slices preincubated with ^{3}H-GABA appeared to be reduced by 50 μM EKC although this did not reach statistical significance. 100 μM EKC failed to alter the KCl-evoked ^{3}H-GABA release, as did the μ-receptor agonist FK 33-824 (50 and 100 μM) (Fig. 1).

The KCl-evoked release of radioactivity from pallidal slices preincubated with ^{3}H-dopamine was increased by exposure of the tissue to the k-opioid receptor agonist EKC (50 and 100 μM) (Fig. 3). In contrast neither the δ-opioid receptor agonist DADLE (50 and 100 μM), or the μ-opioid receptor agonist FK 33-824 (50 and 100 μM), had any effect on the KCl-evoked release of ^{3}H-dopamine (Fig. 2).

The KCl-evoked release of radioactivity from pallidal slices preincubated with ^{3}H-5HT was unaltered by exposure of the tissue to the k-opioid receptor agonist EKC (50 and 100 μM). The KCl-evoked release of radioactivity was similarly unaltered by exposure of pallidal slices to either

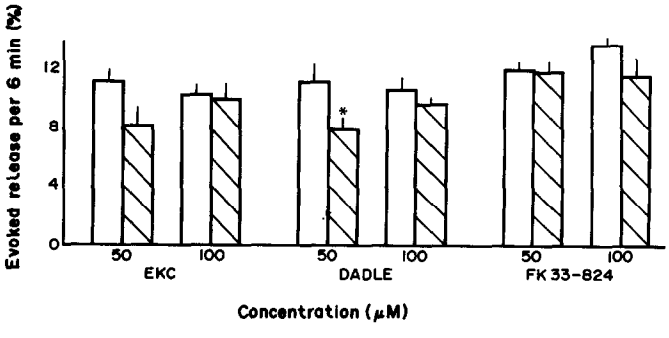

Fig. 1. The effect of EKC (50 and 100 μM); DADLE (50 and 100 μM) and FK 33-824 (50 and 100 μM) on the 25 mM KCl-evoked release of radioactivity from pallidal slices preincubated with ^{3}H-GABA. The results are expressed as the release of radioactivity evoked in the three fractions (6 min) immediately following the addition of KCl. Histograms represent the mean evoked release of radioactivity ±1 SEM. Open columns represent the evoked release of radioactivity from pallidal slices when KCl alone was included in the perfusing buffer. Hatched columns represent the evoked release of radioactivity from pallidal slices when both KCl and opioid agonist drug were included in the perfusing buffer. *$P < 0.05$, Student's *t*-test, N = 6 for each manipulation.

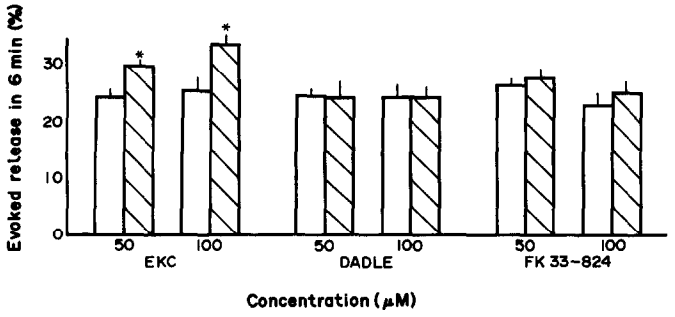

Fig. 2. The effect of EKC (50 and 100 μM); DADLE (50 and 100 μM) and FK 33-824 (50 and 100 μM) on the 25 mM KCl-evoked release of radioactivity from pallidal slices preincubated with ^{3}H-dopamine. The results are expressed as the release of radioactivity evoked in the three fractions (6 min) immediately following the addition of KCl. Histograms represent the mean evoked release of radioactivity ±1 SEM. Open columns represent the evoked release of radioactivity from pallidal slices when KCl alone was included in the perfusing buffer. Hatched columns represent the evoked release of radioactivity from pallidal slices when both KCl and opioid agonist drug were included in the perfusing buffer. *$P < 0.05$, Student's t-test, $N = 6$ for each manipulation.

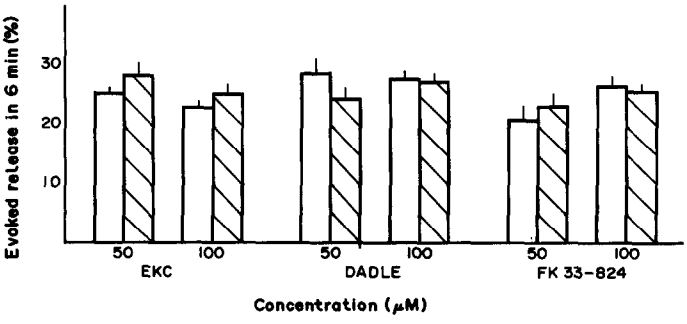

Fig. 3. The effect of EKC (50 and 100 μM); DADLE (50 and 100 μM) and FK 33-824 (50 and 100 μM) on the 25 mM KCl-evoked release of radioactivity from pallidal slices preincubated with ^{3}H-5HT. The results are expressed as the release of radioactivity evoked in the three fractions (6 min) immediately following the addition of KCl. Histograms represent the mean evoked release of radioactivity ± 1 SEM. Open columns represent the evoked release of radioactivity from pallidal slices when KCl alone was included in the perfusing buffer. Hatched columns represent the evoked release of radioactivity from pallidal slices when both KCl and opioid agonist drug were included in the perfusing buffer. $N = 6$ for each manipulation.

the δ-opioid receptor agonist DADLE ((50 and 100 μM) or the μ-opioid receptor agonist FK 33-824 (50 and 100 μM) (Fig. 3).

DISCUSSION

The pallidal slices used were able to accumulate ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT. In the superfusion system employed there was spontaneous release of ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT from prelabelled slices of rat globus pallidus, and calcium-dependent KCl-evoked release of all three substances was demonstrated.

The δ- and μ- and k-opioid receptor agonists, DADLE, FK 33-824 and EKC respectively, did not alter the spontaneous release of ^{3}H-GABA, ^{3}H-dopamine or ^{3}H-5HT from pallidal slices. These results suggest that the opioid agonist drugs cannot actively stimulate neurotransmitter release. In contrast, the opioid agonist drugs did affect the KCl-evoked release of neurotransmitters from prelabelled pallidal slices. The k-opioid receptor agonist, EKC, increased the rate of KCl-evoked release of ^{3}H-dopamine suggesting the presence of K-opioid receptors on the terminals of dopaminergic neurones in the globus pallidus. Neither DADLE (δ-agonist) nor FK 33-824 (μ-agonist) altered the KCl-evoked release of ^{3}H-dopamine. However, DADLE, only at the lowest concentration, decreased the rate of KCl-evoked release of ^{3}H-GABA from prelabelled pallidal slices, suggesting the presence of δ-receptors on the terminals of GABAergic neurones in the globus pallidus. Although 50 μM EKC appeared to reduce the KCl-evoked release of ^{3}H-GABA this was not statistically significant and FK 33-824 had no effect on the release of ^{3}H-GABA. In the nigrostriatal system, opioid receptors occur on the terminals of dopamine neurones [16, 17] and dopamine release from striatal slices was increased by δ-, but not by μ-receptor agonists [18]. Finally, the KCl evoked release of ^{3}H-5HT from prelabelled pallidal slices was unaltered by EKC, DADLE or FK 33-824.

The effect of EKC on KCl-evoked ^{3}H-dopamine release can perhaps explain the results of behavioural experiments.

Unilateral intrapallidal injection of EKC caused ipsiversive circling behaviour [1]. So this may result from increased dopamine release in the globus pallidus leading either directly or indirectly to a reduction in pallidal outflow. Conversely, neither DADLE nor FK 33-824 had any behavioural effect when injected unilaterally into the globus pallidus and similarly they had no effect on ^{3}H-dopamine release from pallidal slices. However, DADLE (50 μM only) decreased the rate of KCl-evoked release of ^{3}H-GABA from prelabelled pallidal slices. This may explain the behavioural consequence of bilateral intrapallidal injections of DADLE, which is to increase locomotor activity [1].

However, it remains puzzling as to why the higher dose of DADLE was without effect on the KCl-evoked release of ^{3}H-GABA. For some reason perhaps high doses of opioid agonist drugs have no effect in this type of release experiment. This may explain the ineffectiveness of FK 33-824 in this work. This drug is 100 times more potent than DADLE in increasing locomotor activity following bilateral intrapallidal injection. Perhaps much lower concentrations of FK 33-824 might have an effect. DADLE also has some μ-receptor actions and so the possibility that these receptors, as well as δ-receptors, are involved in the modulation of GABA release cannot be ruled out. The failure of any of the opioid agonist drugs to alter the release of ^{3}H-5HT from prelabelled pallidal slices suggests that opioid receptors are not located on 5HT terminals in the globus pallidus.

These preliminary findings suggest that different opioid agonist drugs can modulate neurotransmitter release within the globus pallidus. It remains to be established if these effects on pallidal release are due to an action on opioid receptors by investigating the effects of the opioid antagonists.

Acknowledgements—This study was supported by the Parkinson's Disease Society, the Medical Research Council and the Research Funds of the Bethlem Royal and Maudsley Hospitals, and King's College Hospital. We are grateful to Sandoz for gifts of FK 33-824, the Wellcome Research Laboratories for BW 180C, and Sterling Winthrop for ethylketocyclazocine.

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Biochemical Pharmacology, Vol. 36, No. 10, pp. 1741–1747, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
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Arachidonic acid monooxygenase and lipoxygenase activities in polymorphonuclear leukocytes

(*Received* 5 *June* 1986; *accepted* 7 *November* 1986)

Polymorphonuclear leukocytes (PMNs) metabolize arachidonic acid (AA) via several distinct enzymatic pathways. The 5-lipoxygenase enzyme is responsible for the production of 5-hydroxyeicosatetraenoic acid (5-HETE) and 5,12-diHETE (LTB_4) [1]. The formation of 12- and 15-HETE by 12- and 15-lipoxygenases, respectively, has also been described [2, 3]. AA metabolism in PMNs of some species also proceeds via the cyclooxygenase pathway to produce thromboxane A_2 [4, 5], although this activity is not detectable in human cells. The profile of metabolites formed could be stimulus dependent since the calcium ionophore, A23187, appears to selectively stimulate the 5-lipoxygenase [1], whereas bradykinin activates only the 15-lipoxygenase [6]. More recently, we have demonstrated that canine PMNs metabolize AA by a mechanism independent of either the cyclooxygenase or lipoxygenase pathways [7]. The products, called peak 1 (P1) and peak 2 (P2) pending structural analysis, are formed by a cytochrome