DOPAMINE AND [D-ALA², D-LEU⁵]ENKEPHALIN INHIBIT THE ELECTRICALLY STIMULATED NEUROHYPOPHYSEAL RELEASE OF VASOPRESSIN *IN VITRO*: EVIDENCE FOR CALCIUM-DEPENDENT OPIATE ACTION¹

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Received July 14, 1981; Revised September 17, 1981; Accepted September 18, 1981

Abstract

The effects of both dopamine and enkephalin agonists were assessed on electrically stimulated vasopressin secretion from the isolated neural lobe. Both dopamine (10^{-6} M) and the dopamine agonist ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; 10^{-6} M) significantly reduced the stimulated release of vasopressin. This reduction in vasopressin secretion was prevented by the dopamine antagonist sulpiride (10^{-6} M). [D-Ala², D-Leu⁵]Enkephalin (DADLE) also inhibited vasopressin release and its effect was additive to that of ADTN. The effect of DADLE was prevented by the opiate antagonist naloxone and was also sensitive to the calcium content of the medium. Low calcium concentrations markedly increased the inhibitory effect of DADLE, while high concentrations abolished its effect. These findings provide evidence of a direct inhibitory action of both dopamine and enkephalins at the level of the neural lobe and suggest that the effect of enkephalins may be intimately related to calcium-sensitive neurosecretory events.

The neural lobe of the pituitary contains opiate binding sites (Simantov and Snyder, 1977) and also has been shown to contain both opioid peptides (Rossier et al., 1980) and dopamine (Björklund et al., 1973). There is evidence for an inhibitory effect of dopamine on arginine vasopressin secretion *in vivo* (Lightman and Forsling, 1980a) and evidence was obtained previously for a direct inhibitory effect of opioid peptides on vasopressin release from neural lobe *in vitro* (Iversen et al., 1980). Stimulation of vasopressin release by water deprivation also has been shown to result in an increased pituitary concentration of dopamine (Holzbauer et al., 1980) but a markedly diminished pituitary concentration of enkephalins (Rossier et al., 1980).

The present studies investigate the direct effects of dopamine and opiate agonists at the level of the neural lobe. Since studies in other tissues have suggested an important role for calcium in the mediation of opioid actions (Mudge et al., 1979; Guerrero-Munoz et al., 1979), further studies were undertaken to assess whether external calcium concentration was an important factor in the action of opiate agonists.

¹ S. L. was supported by a grant from the Wellcome Trust. We thank K. Todd for her technical assistance.

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Materials and Methods

Adult male Sprague-Dawley rats (180 to 250 gm) were killed by cervical dislocation and the neurointermediate lobes were dissected out. The pituitary stalk was drawn into a silver wire suction electrode as previously described (Iversen et al., 1980) and incubated at 37°C in 0.5 ml of Krebs bicarbonate solution continuously gassed with 95% O₂, 5% CO₂. The medium was changed at 15-min intervals and, following 60 min equilibration, the gland received the first of two periods of electrical stimulation. During these periods of stimulation, the tissue was raised to the surface of the Krebs solution.

Electrical stimulation consisted of 10-sec trains of 40 V given at 30 Hz with a duration of 2 msec. There was a 10-sec interval between trains, and stimulation continued for 10 min. The neural lobes then were lowered deep into the medium and a further 5-min equilibration occurred before the 15-min change of medium was performed.

Test compounds were added to the medium both for the 15 min before stimulation and during the 15-min stimulation period. In all experiments, half of the pituitaries had the test compound added before the first period of stimulation and half before the second period. There was a period of 60 min between the two stimulations.

The perfusing medium was assayed for arginine vaso-

pressin by direct radioimmunoassay (Lightman and For-sling, 1980a).

All results are the mean of a minimum of six experiments and are expressed $\pm$ SD unless otherwise stated. Statistical analysis was performed using the Student's *t* test for unpaired data.

Results

Stimulation of the neural lobe of the pituitary resulted in a very marked increase in vasopressin release (Fig. 1). The vasopressin released into the incubation medium rose from a mean basal value of 0.17 milliunit during the 15-min period prior to stimulation to 3.56 milliunits during the stimulation period. An increased release of vasopressin also was found during the following 15-min period, and the stimulated release was calculated by the addition of the vasopressin released during both the stimulation period and the 15 min following stimulation.

Calcium dependence of vasopressin release. The effect of changing the external calcium concentration ($[Ca]_0$) is shown in Figure 2. Reduction of $[Ca]_0$ produced a progressive fall in the electrically stimulated release of vasopressin and, thus, in the $S_t:S_c$ ratio. The calcium antagonist verapamil also inhibited vasopressin release, an effect that was exaggerated at low $[Ca]_0$.

Effect of dopamine, enkephalin, and cholinergic agents. The effects of these agents are summarized in Table I. Both dopamine and the rigid dopamine agonist ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) produced small but significant reductions ($p < 0.01$) in electrically stimulated vasopressin release. The response to ADTN was prevented by the dopamine antagonists sulpiride and domperidone, neither of which influenced vasopressin release when given alone.

The enkephalin analogue DADLE ($[D-Ala^2, D-Leu^5]$ -enkephalin) also reduced vasopressin release ($p < 0.001$), an effect which could be prevented by the simultaneous addition of naloxone. DADLE and ADTN together had an additive effect on the inhibition of vasopressin release.

Nicotine, acetylcholine, and hexamethonium were tested for an effect on electrically stimulated vasopressin release. None of these agents had any significant effect.

Role of calcium in the response to DADLE. Reduction of $[Ca]_0$ resulted in a greater inhibition of vasopressin release in response to DADLE (Fig. 3). A similar enhanced effect was produced by the addition of verapamil to the medium. An increase of $[Ca]_0$ to 6.5 mM, however, abolished the inhibitory effect of DADLE.

Figure 1. Release of vasopressin from the isolated neural lobe. Electrical stimulation was given in 10-sec trains of 40 V at 30 Hz with a duration of 2 msec. There were 10-sec intervals between trains and stimulation continued for 10 min. The values represent the total arginine vasopressin (AVP) released per lobe for 15-min incubations before, during, and after the 10-min period of stimulation. Mean $\pm$ SEM; $n = 20$. mU, milliunits.

Figure 2. Calcium dependence of electrically stimulated vasopressin release. The external calcium concentration was altered or the calcium antagonist verapamil was added during the test stimulation period S_t . Values represent the mean $\pm$ SD for a minimum of six experiments. *, $p < 0.02$; **, $p < 0.01$; ***, $p < 0.001$. S_c , control stimulation.

TABLE I
Effect of test compounds on the electrically stimulated release of vasopressin

Test compounds were added to the incubation medium both for the 15 min before stimulation and during the 15-min incubation period when the test stimulation (S_t) was applied to the neural lobe. Results are expressed as a ratio of the vasopressin released during this test stimulation to the vasopressin released during a control stimulation period (S_c) when no test compounds were present. Each ratio represents the mean of a minimum of six experiments.

Test Compound		$S_t:S_c$ Ratio (Mean $\pm$ SD)	p (Compared with Control)
Control		0.97 ± 0.17	
Dopamine	1 μM	0.73 ± 0.16	<0.01
ADTN	1 μM	0.71 ± 0.14	<0.01
Sulpiride	1 μM	1.01 ± 0.21	ns ^a
Domperidone	1 μM	1.02 ± 0.31	ns
ADTN	1 μM + sulpiride 1 μM	0.89 ± 0.17	ns
ADTN	1 μM + domperidone 1 μM	0.93 ± 0.20	ns
DADLE	1 μM	0.80 ± 0.19	ns
DADLE	5 μM	0.66 ± 0.12	<0.001
Naloxone	1 μM	1.09 ± 0.21	ns
DADLE	5 μM + naloxone 1 μM	0.92 ± 0.24	ns
DADLE	5 μM + ADTN 1 μM	0.50 ± 0.08	<0.001
Nicotine	10 μM	1.12 ± 0.26	ns
Acetylcholine	10 μM	0.90 ± 0.19	ns
Hexamethonium	10 μM	0.96 ± 0.16	ns

^a ns, not significant.

S_t & S_c : Ca^{2+} 2.6 mM Ca^{2+} 2.6 mM Ca^{2+} 0.26 mM Verapamil 50 μM Ca^{2+} 6.5 mM
 S_t only: — DADLE 5 μM DADLE 5 μM DADLE 5 μM DADLE 5 μM

Figure 3. Calcium-dependent change of neural lobe sensitivity to 5 μM DADLE. The external calcium concentration was altered or verapamil was added during both the control stimulation S_c and the test stimulation period S_t . Values represent the mean $\pm$ SD for a minimum of six experiments. $\times\times\times$, $p < 0.001$.

The effect of changes in $[\text{Ca}]_0$ on inhibition of vasopressin release by ADTN (1 μM) also was investigated. Reduction of $[\text{Ca}]_0$ to 0.26 mM during both S_c and S_t resulted in an $S_t:S_c$ ratio of 0.68 ± 0.19 , while an increase of $[\text{Ca}]_0$ to 6.5 mM gave a ratio of 0.76 ± 0.15 . Neither ratio was significantly different from the control study with 2.6 mM $[\text{Ca}]_0$ ($S_t:S_c$ ratio, 0.71 ± 0.14).

Discussion

Immunoassay and histochemical studies have confirmed the presence of both opioid peptides (Rossier et al., 1980) and fine varicose dopaminergic nerve fibers (Baumgarten et al., 1972; Björklund et al., 1973) in the neural lobe. *In vivo* studies have produced confusing data on the role of these compounds in the control of neurohypophyseal function. Opiates have been shown to have either stimulatory or inhibitory effects on vasopressin

secretion depending on the dose, the route of administration, and the time when observations are made (Lightman and Forsling, 1980b; Aziz et al., 1981). Similarly, dopamine administered in different ways into different species has been shown both to increase and to diminish vasopressin release (Lightman and Forsling, 1980a).

The present studies were designed to define the effects of dopaminergic opiate and cholinergic agonists at the level of the neural lobe. Opioid peptides do not alter spontaneous release of vasopressin from isolated neurointermediate lobes (Weitzman et al., 1977). We have, however, confirmed the earlier finding of a naloxone-sensitive direct inhibitory effect of opiates on electrically stimulated release (Iversen et al., 1980). In addition, we find that dopamine itself results in a significant inhibition of electrically stimulated vasopressin secretion. This is a dopaminergic rather than adrenergic effect since it also

occurs with the dopamine agonist ADTN and is inhibited by the D2 dopamine inhibitors sulpiride and domperidone.

Cholinergic agents which have been widely shown to increase vasopressin release *in vivo* (Kuhn, 1974) and also have been shown to be active in the organ-cultured hypothalamo-neurohypophyseal preparation (Sladek and Knigge, 1977) had no direct effects at the level of the neural lobe.

These data suggest that both opiate and dopaminergic, but not cholinergic, receptors are present at preterminal sites on vasopressin neurosecretory fibers in the neurohypophysis. This would explain why the decrease in vasopressin release following parenteral administration of L-dopa is prevented by inhibition of its conversion to dopamine in the pituitary gland (Lightman and Forsling, 1980a). Also, the direct inhibitory effect of opiates at the level of the neural lobe probably represents the locus at which small doses of intracerebroventricular morphine inhibit vasopressin secretion (Van Wimersma Griedanus et al., 1979; Aziz et al., 1981).

The changes of neurohypophyseal sensitivity to DADLE following alterations in the calcium concentration of the perfusate suggest that calcium fluxes play a critical role in mediating DADLE's inhibitory effect. Enkephalins have been shown previously to decrease both the duration and magnitude of the Ca^{2+} component of the soma spike in dorsal root ganglia (Mudge et al., 1979), and morphine has been shown to reduce $^{45}\text{Ca}^{2+}$ uptake into mouse brain synaptosomes (Guerrero-Munoz et al., 1979). Our results suggest that calcium fluxes may mediate the actions of opioid peptides in neurohypophyseal neurosecretion.

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