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Studies on the adrenomedullary dependence of κ-opioid agonist-induced diuresis in conscious rats

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1 The dependence of κ-opioid agonist-induced diuresis, upon an intact and functional adrenal medulla in conscious rats, was investigated in order to test the hypothesis that the diuresis is mediated by a blood-borne 'diuretic factor', of adrenomedullary origin, released by κ-opioid receptor stimulation.

2 Confirming previous observations, adrenal demedullation significantly attenuated diuretic responses to the κ-opioid agonists U50488H, ethylketocyclazocine (EKC) and tifluadom, but did not affect basal urine output, furosemide-induced diuresis or the antidiuretic response to the μ-opioid agonist, buprenorphine. Naloxone abolished U50488H-induced diuresis, confirming an involvement of opioid receptors.

3 Transfusion studies established that blood, from intact rats treated with U50488H, induced diuresis in intact and demedullated recipient rats, whether or not the recipients had been pretreated with naloxone. However, blood from demedullated rats treated with U50448H was unable to induce diuresis when administered to intact or demedullated recipients.

4 It is concluded that κ-opioid agonist-induced diuresis is dependent upon an intact and functional adrenal medulla and appears to be mediated by a blood-borne 'diuretic factor' of adrenomedullary origin.

Introduction

An opioid receptor-mediated suppression of the neurohypophysial release of vasopressin was considered to be responsible for the water diuresis induced by κ-opioid agonists in normally hydrated rats (Leander, 1983a,b), although Miller (1975) had shown vasopressin release to be inhibited by non-selective narcotic antagonists. The identification of opioid receptors (Castanas *et al.*, 1984) and immunoreactivity to vasopressin (Ang & Jenkins, 1984) in the adrenal medulla, raised the possibility that the diuretic response to κ-opioid agonists might involve a peripheral component, dependent upon an intact and functional adrenal medulla (Blackburn *et al.*, 1985; 1986). Indeed, adrenal demedullation was found to abolish selectively the diuretic response to the κ-opioid agonists, U50488H, ethylketocyclazocine (EKC) and tifluadom (Blackburn *et al.*, 1986).

This adrenomedullary dependence of κ-opioid agonist-induced diuresis, led to the suggestion that κ-opioids may be capable of stimulating the release of an, as yet unidentified, 'diuretic factor' of adrenomedullary origin. The present transfusion experiments were performed in an attempt to establish the existence of the postulated 'diuretic factor'.

Methods

Animals

Male Wistar rats (175–200 g body weight), obtained from Charles River, Montreal, were used throughout. Under Saffan (alphaxolone/alphadolone; 12 mg kg^{-1}, i.v.) anaesthesia, bilateral adrenal demedullation was performed by methods described previously (Borkowski & Quinn, 1983). Seven days later, a carotid artery and a jugular vein were catheterized, under Saffan anaesthesia, to facilitate arterial blood sampling and intravenous drug administration respectively. The rats were used in experiments not less than 48 h after catheter implantation. To minimize post-operative discomfort, all animals were treated with Talwin (pentazocine; 10 mg kg^{-1}, s.c.) in the immediate post-operative period. In accord with the previous study (Blackburn *et al.*, 1986), preliminary observations established that sham adrenal

demedullated rats exhibited plasma catecholamine levels and urinary responses to U50488H, tifluadom, EKC, furosemide and buprenorphine, which were similar to those obtained in catheterized but otherwise unoperated control animals. Thus, all further experiments were performed on catheterized rats with (intact) and without (demedullated) adrenal medullae.

Diuretic studies

Intact and demedullated animals were given a saline load (0.9% w/v NaCl; 10 ml kg^{-1}, p.o.) and placed in individual Nalge metabolism cages (Fisher Scientific) for rodents. The arterial and venous catheters were connected to 1 ml syringes via polyethylene tubing (PE50) filled with heparinized saline. These enabled arterial blood sampling and intravenous drug administration without disturbing the rats during the experiment.

The urine collected in the first 30 min after saline-loading was measured and discarded. At this time, test drugs or saline were administered intravenously, in a volume of 1 ml kg^{-1}, and flushed-in with 0.2 ml of heparinized saline. Antagonists were administered in a similar manner 5 min before the agonists. The volume of urine collected in the subsequent 4 h was expressed in terms of:- ml h^{-1} 100 g^{-1} body weight.

Transfusion studies

Donor rats were saline-loaded and the urine collected in the first 30 min discarded. The donors were then given the test drug intravenously and the recipient rats saline-loaded. Thirty minutes later, the urine collected from recipients was discarded, 1 ml of arterial blood was drawn from the donor rats (over 30 s) and immediately given intravenously to the recipients (again over 30 s). The blood withdrawn from the donors was replaced with 1 ml of saline and this, and the blood infused into the recipients, was flushed-in with 0.2 ml of heparinized saline. Antagonists were injected 5 min before the agonists in donor rats and 5 min before the blood transfusion in recipient animals. The rats remained in the metabolism cages until 4 h had elapsed from the time of drug/donor blood administration.

Catecholamine assays

The effectiveness of adrenal demedullation was determined by measuring plasma and adrenal gland catecholamine content in 6 intact and 6 demedullated rats, chosen at random from the experimental groups, before they had been subjected to any drug treatment. Forty eight hours after catheterization (i.e. 9 days after demedullation), the rats were placed in metabolism cages. One hour later, 1 ml of blood was collected, by free-flow, into ice-cold centrifuge tubes containing heparin (20 units in 20 μl). The red cells were spun down (3000 g × 10 min at 4°C) and the plasma catecholamines extracted onto activated alumina and eluted with 0.1 M perchloric acid, according to a modification of the method of Anton & Sayre (1962). Catecholamine concentration was measured by reverse-phase high performance liquid chromatography with electrochemical detection (h.p.l.c.-e.d.). Two days after blood sampling, the rats were anaesthetized with Saffan, the adrenal glands removed and homogenized in 1 ml aliquots of 0.1 M perchloric acid containing a trace (400 $\mu l\,l^{-1}$) of 1 M $NaHSO_3$. The homogenates were centrifuged (3000 g × 10 min), the supernatant filtered through 0.22 μm pore-size filters to remove colloidal protein, and assayed by h.p.l.c.-e.d.

Drugs

Generous donations of the following drugs are gratefully acknowledged:- U50488H (trans-3,4-dichloro-N-methyl-N-[2-(pyrrolidinyl)-cyclohexyl]-benzeneacetamide methane sulphonate) (Upjohn), tifluadom (Sandoz), EKC (Winthrop) and buprenorphine (Schering). Furosemide and naloxone were obtained from Sigma. All drugs were dissolved freshly in saline, with the aid of minimal amounts of dilute HCl or gentle warming if necessary, given intravenously in a volume of 1 ml kg^{-1} and flushed-in with 0.2 ml of heparinized saline. Saline was used as the control. Experimental groups consisted of a minimum of 6 animals and data are presented as the mean ± standard error of the mean (s.e.mean), where n is the number of observations. Statistical analysis was by Student's unpaired t test and significance was accepted when $P < 0.05$.

Results

Diuretic studies in intact and adrenal demedullated rats

Nine days after bilateral adrenal demedullation, plasma adrenaline levels were reduced by 93% compared to intact rats and by 92% compared to sham-operated animals (intact: 492 ± 86 pg ml^{-1}; sham-operated: 451 ± 99 pg ml^{-1}; demedullated: 34 ± 7 pg ml^{-1}, $n = 6$ in all cases). Demedullation-induced changes in plasma noradrenaline and dopamine levels were small and did not achieve statistical significance. Eleven days after demedullation, adrenal gland noradrenaline (64.5 ± 6.3 ng) and adrenaline (90.5 ± 22.3 ng) content, per pair of glands, was reduced by more than 99% compared to levels in intact (9.9 ± 1.4 μg and 25.5 ± 3.8 μg, respectively) or sham-operated (10.4 ± 2.1 μg and 24.9 ± 3.0 μg) rats.

In keeping with earlier observations (Blackburn *et al.*, 1986) in the Alderley Park Wistar rat, there was

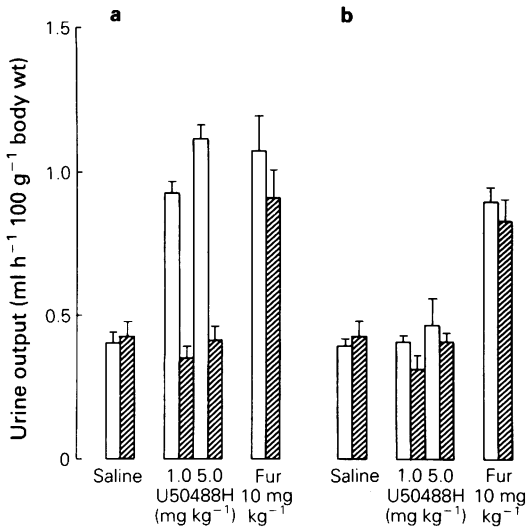

Figure 1 Effect of pretreatment with naloxone (10 mg kg^{-1}, i.v.) on the cumulative urine output over 4 h, expressed in terms of:- ml h^{-1} 100 g^{-1} body weight, induced by saline (1 ml kg^{-1}, i.v.), furosemide (Fur; 10 mg kg^{-1}, i.v.) and U50488H (1–5 mg kg^{-1}, i.v.) in conscious saline (10 ml kg^{-1}, p.o.)-loaded intact (a) and adrenal demedullated (b) rats. Effects in saline (1 ml kg^{-1}, i.v.) pretreated controls are indicated by the open columns. The hatched columns indicate urine output in naloxone pretreated rats. Each column represents the mean of a group of 8 rats and the vertical lines indicate s.e.mean.

no significant difference in basal urine output between intact (0.407 ± 0.038 ml h^{-1} 100 g^{-1} body weight, $n = 16$), sham-operated (0.449 ± 0.058 ml h^{-1} 100 g^{-1} body weight, $n = 8$) and adrenal demedullated (0.393 ± 0.026 ml h^{-1} 100 g^{-1} body weight, $n = 16$) conscious, saline-loaded Wistar rats. Nor did adrenal demedullation significantly affect the diuretic response (1.080 ± 0.120 ml h^{-1} 100 g^{-1} body weight, $n = 8$) to furosemide (10 mg kg^{-1}, i.v.) or the antidiuretic response (0.165 ± 0.035 ml h^{-1} 100 g^{-1} body weight, $n = 8$) to buprenorphine (1 mg kg^{-1}, i.v.). However, as was the case in the previous study (Blackburn *et al.*, 1986), the diuretic responses to U50488H (1 mg kg^{-1}, i.v.), tifluadom (5 mg kg^{-1}, i.v.) and EKC (1 mg kg^{-1}, i.v.) were attenuated in demedullated rats (0.430 ± 0.030 ml h^{-1} 100 g^{-1} body weight, 0.600 ± 0.055 ml h^{-1} 100 g^{-1} body weight and 0.510 ± 0.050 ml h^{-1} 100 g^{-1} body weight, respectively; $n = 8$ in all cases) when compared to those obtained in intact animals (0.930 ± 0.040 ml h^{-1} 100 g^{-1} body weight, 0.855 ± 0.065 ml h^{-1} 100 g^{-1} body weight and 1.000 ± 0.115 ml h^{-1} 100 g^{-1} body weight, respectively; $n = 8$ in all cases).

Table 1 Urine output over 4 h, (ml h^{-1} 100 g^{-1} body weight) in conscious intact donor and recipient rats

Group	*Donors*	*Recipients*
1 ($n = 7$)	Intact + saline	Intact
	0.374 ± 0.093	0.464 ± 0.114
2 ($n = 7$)	Intact + U50488H	Intact
	0.871 ± 0.079[a]	0.757 ± 0.062[b]
3 ($n = 6$)	Intact + U50488H	Intact + Nal
	0.897 ± 0.099[a]	0.810 ± 0.102[b]
4 ($n = 6$)	Intact + Nal + U50488H	Intact
	0.413 ± 0.054[c]	0.450 ± 0.050[d]
5 ($n = 6$)	Intact + Nal + U50488H	Intact + Nal
	0.455 ± 0.054[c]	0.473 ± 0.041[e]

Thirty min after saline loading, recipient animals received a 1 ml blood transfusion from donor rats which had been given test drugs 30 min previously. The test drugs administered were:- saline (1 ml kg^{-1}, i.v.); U50488H (5 mg kg^{-1}, i.v.) and naloxone (Nal; 10 mg kg^{-1}, i.v.). U50488H and naloxone were administered in a volume of 1 ml kg^{-1} and all injections were flushed in with 0.2 ml of heparinized saline.

[a] $P < 0.01$, comparing donor groups 2 or 3 to group 1.

[b] $P < 0.05$, comparing recipient groups 2 or 3 to group 1.

[c] $P < 0.01$, comparing donor groups 4 or 5 to groups 2 or 3.

[d] $P < 0.01$, comparing recipient group 4 to group 2.

[e] $P < 0.05$, comparing recipient group 5 to group 3.

There were no significant differences between donors and recipients in any one experimental group. All analyses were performed by Student's unpaired *t* test.

Transfusion studies

The agonist U50488H, which is highly selective for κ-opioid receptors (James & Goldstein, 1984), was chosen for use in the transfusion studies. A dose of 5 mg kg^{-1} U50488H (i.v.) was selected since it induced a marked but submaximal diuresis, in intact rats, which could be abolished by naloxone (10 mg kg^{-1}, i.v.) pretreatment and was attenuated significantly in adrenal demedullated rats (Figure 1; Tables 1 and 2).

Naloxone (10 mg kg^{-1}, i.v.) pretreatment did not affect basal urine output nor furosemide (10 mg kg^{-1}, i.v.)-induced diuresis (Figure 1), indicating a selective action against U50488H. The ability of naloxone to block the diuretic effects of U50488H, together with the lack of a diuretic response to control injections of saline (1 ml kg^{-1}, i.v.), also indicates that U50488H-induced diuresis is not a volume effect.

Transfusing 1 ml of blood, from an intact donor rat which had received saline (1 ml kg^{-1}, i.v.) 30 min previously, into an intact recipient, did not affect urine output in the recipient animal (Table 1), again indicating a lack of volume effect of the transfused blood. However, transfusing 1 ml of blood, from an intact donor which had received U50488H (5 mg kg^{-1}, i.v.) 30 min previously, into an intact recipient, induced a diuretic response which was smaller but not significantly different from that induced by U50488H in the donor rat (Table 1). This diuretic response in the recipient was not due to U50488H in the donor blood, since pretreating the recipient with naloxone (10 mg kg^{-1}, i.v.), a dose which abolished U50488H (5 mg kg^{-1}, i.v.) induced diuresis, did not attenuate the diuretic response induced by the transfused blood in the recipient rat (Table 1).

Pretreating intact donor rats with naloxone (10 mg kg^{-1}, i.v.) not only abolished the diuretic response to the subsequent injection of U50488H (5 mg kg^{-1}, i.v.) in these animals (Table 1), but the blood from these donors no longer induced diuresis in intact recipients, whether or not the recipient rats had been pretreated with naloxone (Table 1). This observation supports the view that the contents, and not the volume, of the transfused blood induce diuresis in the recipient animals.

Despite there being no difference between demedulated and intact rats, when comparing their basal urine output and urinary responses to intravenous saline, furosemide (Figure 1; Tables 1 and 2) and buprenorphine, U50488H (5 mg kg^{-1}, i.v.) was no longer able to induce a diuretic response in conscious rats which had undergone bilateral adrenal demedullation 9 to 14 days previously (Figure 1; Table 2).

Transfusing 1 ml of blood from demedullated donor rats, which had received either saline (1 ml kg^{-1}, i.v.) or U50488H (5 mg kg^{-1}, i.v.) 30 min previously, failed to induce a diuretic response in either intact or demedullated recipients (Table 2) and offers further support to the contention that the volume of transfused blood is without diuretic effect. This also indicates that 1 ml of blood transfused, from a donor rat treated with U50488H, contains insufficient U50488H to induce diuresis in a recipient.

Blood transfused from intact donors treated with saline (1 ml kg^{-1}, i.v.) did not affect urine output in demedullated recipients (Table 2). However, blood from intact donors treated with U50488H (5 mg kg^{-1}, i.v.) induced diuresis in demedullated recipients (Table 2). This diuretic response was significantly smaller than that induced by a similar transfusion or the direct injection of U50488H (5 mg kg^{-1}, i.v.) in intact rats, but was significantly greater than the urine output observed in demedullated rats treated with U50488H (5 mg kg^{-1}, i.v.) and in demedullated recipients of blood from either intact donors treated with saline or demedullated donors treated with U50488H (Tables 1 and 2).

Table 2 Urine output over 4 h (ml h^{-1} 100 g^{-1} body weight) in conscious intact and adrenal demedullated (Demed) donor and recipient rats

Group	*Donors*	*Recipients*
1 ($n = 7$)	Demed + saline	Demed
	0.389 ± 0.068	0.440 ± 0.065
2 ($n = 6$)	Demed + U50488H	Demed
	0.466 ± 0.093	0.444 ± 0.068
3 ($n = 6$)	Demed + U50488H	Demed + Nal
	0.444 ± 0.030	0.391 ± 0.031
4 ($n = 6$)	Demed + saline	Intact
	0.396 ± 0.034	0.455 ± 0.041
5 ($n = 6$)	Demed + U50488H	Intact
	0.420 ± 0.058	0.506 ± 0.053
6 ($n = 6$)	Intact + saline	Demed
	0.440 ± 0.059	0.416 ± 0.075
7 ($n = 6$)	Intact + U50488H	Demed
	0.962 ± 0.147[a,d]	0.628 ± 0.052[b]
8 ($n = 6$)	Intact + U50488H	Demed + Nal
	0.886 ± 0.092[a,d]	0.604 ± 0.080[c]

Thirty min after saline loading, recipient rats received a 1 ml blood transfusion from donor animals which had been given test drugs 30 min previously.
Test drugs and their administration was the same as in Table 1.
[a] $P < 0.01$, comparing donor groups 7 or 8 to groups 2, 3, 5 or 6.
[b] $P < 0.05$, comparing recipient group 7 to groups 2 or 6.
[c] $P < 0.05$, comparing recipient group 8 to group 3.
[d] $P < 0.05$, comparing donor groups 7 and 8 to recipient groups 7 and 8 respectively.
All analyses were performed by Student's unpaired *t* test.

Discussion

A previous study (Blackburn *et al.*, 1986) established that bilateral adrenal demedullation selectively abolished the diuretic responses to subcutaneous injections of equieffective doses of the κ-opioid agonists U50488H, tifluadom and EKC in conscious saline-loaded rats. Basal urine output, responses to furosemide, clonidine and the antidiuretic responses to the μ-opioid agonists buprenorphine and morphine were unaffected, indicating the selective nature of adrenal demedullation against κ-opioid agonists.

The present studies employed intravenous drug administration and paralleled these findings, supporting the view that κ-opioid agonist-induced

diuresis involves the adrenal medulla and cannot be explained purely in terms of a central action at the neurohypophysis, since adrenal demedullation would not be expected to have a direct effect at this site. This adrenomedullary dependence lead to the hypothesis that κ-opioid agonist-induced diuresis may involve the release of a 'diuretic factor' of adrenomedullary origin (Blackburn *et al.*, 1986). The present studies sought to confirm earlier observations and attempt to establish the possible existence of the factor. The isolation of the factor, its identification and determination of its mechanism/s of action (e.g. a direct effect on the kidney or an indirect effect via the inhibition of neurohypophysial vasopressin release) have yet to be attempted.

The adrenal medulla contains numerous endogenous transmitters and hormones (Ang & Jenkins, 1984; Lemaire *et al.*, 1984), yet catecholamines comprise the major portion of the adrenomedullary contents (Kirshner, 1972). Thus, the effectiveness of adrenal demedullation was assessed by measuring plasma adrenaline and adrenal gland catecholamine content. In agreement with previous studies (Borkowski & Quinn, 1983; 1985; Blackburn *et al.*, 1986), surgical enucleation significantly reduced plasma adrenaline and adrenal gland catecholamine levels, indicating effective removal of the adrenal medulla. Whilst not measured in the present study, plasma corticosterone is unaffected by demedullation (Borkowski & Quinn, 1983; Blackburn *et al.*, 1986) indicating that the adrenal cortex remains functional and appears not to be involved in the diuretic response to κ-opioid agonists.

The adrenomedullary dependence of κ-opioid agonist-induced diuresis, observed in this and the previous studies (Blackburn *et al.*, 1985; 1986), might be explicable as follows:-

(a) κ-opioid agonists induce diuresis by stimulating opioid receptors, suppressing vasopressin release, in both the neurohypophysis and the adrenal medulla. The medullary opioid receptors are more sensitive than those in the neurohypophysis, so that higher doses of agonist are required to induce diuresis in the absence of the adrenal medulla, or (b) κ-opioid agonists act at the neurohypophysis to suppress the release of vasopressin. Their access to this central site of action is aided by a factor released from the adrenal medulla in response to κ-opioid receptor stimulation. The factor is absent in adrenal demedullated rats which, therefore, require higher systemic doses of the agonists in order to achieve concentrations at the neurohypophysis similar to those in intact rats.

Unfortunately, neither of these possibilities is consistent with the results of the studies which established that the diuretic effect of U50488H was transferable, between rats, by transfusing 1 ml of blood. That this was not a volume effect is indicated by observations that blood, transfused from saline-treated intact or demedullated donor rats, had no effect on urine output in intact or demedullated recipients. Moreover, blood from demedullated donors treated with U50488H failed to induce a diuretic response in either intact or demedullated recipients, supporting the contention that the amount of U50488H, in the blood transfused from a U50488H-treated donor, is insufficient to induce diuresis.

Of still greater interest was the observation that blood, from a U50488H-treated intact donor, induced a diuretic response in both intact and demedullated recipients. The inability of U50488H to induce diuresis in demedullated rats and the inability of naloxone pretreatment of the recipients to attenuate the transfusion-induced diuresis, indicates that the diuretic response in the recipients was not due to any U50488H in the donor blood. The lack of effect of naloxone in the recipients, also indicates that opioid receptors are not involved in mediating the diuretic effects of the transfused blood and suggests that the postulated adrenomedullary 'diuretic factor' is not an opioid.

The data indicate that κ-opioid agonist-induced diuresis is, indeed, dependent upon an intact and functional adrenal medulla.

The transferability of U50488H-induced diuresis between rats, by blood transfusion, indicates that this dependence may be due to the presence of a blood-borne 'diuretic factor' of adrenomedullary origin. That the release of the factor is due to κ-opioid receptor stimulation, is supported by observations that naloxone pretreatment of intact rats not only abolished U50488H-induced diuresis in these animals, but also deprived the blood transfused from them of its ability to induce diuresis in recipient rats. The identity and mechanism/s of action of the 'factor' remain to be elucidated.

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References

ANG, V.T.Y. & JENKINS, J.S. (1984). Neurohypophysial hormones in the adrenal medulla. *J. Clin. Endocrinol. Metab.*, **58**, 688–691.

ANTON, A.H. & SAYRE, D.F. (1962). A study of the factors affecting the aluminium oxide trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmacol. Exp. Ther.*, **138**, 360–375.

BLACKBURN, T.P., BORKOWSKI, K.R. & FRIEND, J. (1985). K-opioid diuretic effects of tifluadom mediated in the periphery? *Br. J. Pharmacol.*, **85**, 382P.

BLACKBURN, T.P., BORKOWSKI, K.R., FRIEND, J. & RANCE, M.J. (1986). On the mechanisms of k-opioid-induced diuresis. *Br. J. Pharmacol.*, **89**, 593–598.

BORKOWSKI, K.R. & QUINN, P. (1983). The effect of bilateral adrenal demedullation on vascular reactivity and blood pressure in spontaneously hypertensive rats. *Br. J. Pharmacol.*, **80**, 429–437.

BORKOWSKI, K.R. & QUINN, P. (1985). Adrenaline and the development of spontaneous hypertension in rats. *J. Autonom. Pharmacol.*, **5**, 89–100.

CASTANAS, E., GIRAUD, P., AUDIGIER, Y., DRISSI, R., BOUDOURESQUE, F., CONTE-DEVOLX, B. & OLIVER, C. (1984). Adrenal medullary opiate receptors. Pharmacological characterization in bovine adrenal medulla and a human phaeochromocytoma. *Molec. Pharmacol.*, **25**, 38–45.

JAMES, I.F. & GOLDSTEIN, A. (1984). Site-directed alkylation of multiple opioid receptors. I. Binding selectivity. *Molec. Pharmacol.*, **25**, 337–342.

KIRSHNER, N. (1972). The adrenal medulla. In *The Structure and Function of Nervous Tissue.* ed. Bourne, G.H. Vol. 5, pp. 163–204. New York: Academic Press.

LEANDER, J.D. (1983a). A kappa opioid effect. Increased urination in the rat. *J. Pharmacol. Exp. Ther.*, **224**, 89–94.

LEANDER, J.D. (1983b). Further study of kappa opioids on increased urination. *J. Pharmacol. Exp. Ther.*, **227**, 35–41.

LEMAIRE, S., DAY, R., DUMONT, M., CHOUINARD, L. & CALVERT, R. (1984). Dynorphin and enkephalins in adrenal paraneurones. Opiates in the adrenal medulla.. *Can. J. Physiol. Pharmacol.*, **62**, 484–492.

MILLER, M. (1975). Inhibition of ADH release in the rat by narcotic antagonists. *Neuroendocrinol.*, **19**, 241–251.

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