Molecular and Cellular Endocrinology, 72 (1990) 95–102
Elsevier Scientific Publishers Ireland, Ltd.

MOLCEL 02330

Effects of suramin on hormone release by cultured rat anterior pituitary cells

Hamdy F.A.I. Marzouk [1,*], Leo J. Hofland [1], Fred H. den Holder [1], Peter M. van Koetsveld [1], Jacobie Steenbergen [2], Joke Zuiderwijk [1], Ekbal M. Abou-Hashim [1,*], Mohammed H. El-Kannishy [1,*], Frank H. de Jong [2] and Steven W.J. Lamberts [1]

Departments of [1] Medicine and [2] Biochemistry (Chemical Endocrinology), Erasmus University, Rotterdam, The Netherlands

(Received 16 March 1990; accepted 21 May 1990)

Key words: Pituitary; Suramin; Hormone; Medium

Summary

Suramin is a polyanionic compound which has been used in the treatment of trypanosomiasis and acquired immunodeficiency syndrome (AIDS), while preliminary success has been reported in the treatment of cancer. However, suramin also causes adrenal insufficiency. We have previously reported that suramin selectively inhibited corticotropin (ACTH)-stimulated corticosterone release by dispersed adrenal cells in a dose-dependent manner via a direct interaction with the ACTH molecule. The present study was undertaken in order to investigate the effect of suramin on hormone release by dispersed rat anterior pituitary cells. Suramin at a concentration of 100 μM inhibited both basal and secretagogue-stimulated ACTH release by cells cultured in minimal essential medium (MEM) only, while it had no effect on ACTH release by cells cultured in MEM + 10% fetal calf serum (FCS) or MEM + 0.1% bovine serum albumin (BSA). In addition, suramin also caused a parallel decrease of prolactin (PRL) and growth hormone (GH) release by cells cultured in MEM only, suggesting a toxic, rather than a selective effect of suramin on anterior pituitary cells cultured in MEM only. In addition, suramin potentiated the effect of thyrotropin-releasing hormone (TRH) on PRL release by cells cultured in MEM + 10% FCS and suppressed the inhibitory effect of dopamine (DA) on PRL release by cells cultured in MEM + 10% FCS and in MEM + 0.1% BSA. Comparable suppressive effects of suramin on growth hormone-releasing hormone (GHRH)-stimulated and somatostatin (SRIH)-inhibited GH release were found in cells cultured in MEM + 0.1% BSA but not in cells cultured in MEM + 10% FCS. Finally, suramin blocked the bovine follicular fluid (bFF)-mediated inhibition of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) release by cells cultured in MEM + 10% FCS, probably via a direct interaction with inhibin in the bFF. From these results we conclude that: (1) the effect of suramin on the hypothalamo–pituitary–adrenal axis appears to be mainly restricted to the adrenals, because its inhibitory effects on ACTH release by dispersed anterior pituitary cells appears to be nonspecific, being prevented by adding 0.1% BSA or 10% FCS. (2) The effect of suramin on PRL and GH release in response to their secretagogues is variable and

Address for correspondence: Prof. S.W.J. Lamberts, Professor of Medicine, Department of Medicine III, University Hospital Dijkzigt, 40 Dr. Molewaterplein, 3015 GD Rotterdam, The Netherlands.

* On leave from the Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt.

dependent on the protein environment of the cells. (3) The effect of suramin on bFF-mediated inhibition of FSH and LH release by pituitary cells seems to be rather specific through an interaction with inhibin and abolishment of its inhibitory effects on FSH and LH release.

Introduction

Suramin sodium, the sodium salt of sulfonated naphthyl polyurea which contains six reactive sulfonic acid carbonyl groups (Fig. 1), was initially introduced for the treatment of African trypanosomiasis (sleeping sickness), onchocerciasis and pemphigus (Wanson, 1950; Aptel, 1970). Being a reverse transcriptase inhibitor (De Clercq, 1979), it has also been evaluated as a potential treatment for viral infections in immunodeficient humans and for the acquired immunodeficiency syndrome (AIDS) (Mitsuya et al., 1984; Broder et al., 1985; De Clercq, 1987). Thereafter it was found that suramin inhibits the binding of platelet-derived growth factor to its receptor (Hosang, 1985; Johnsson et al., 1986). More recently suramin was described as a relatively selective competitive antagonist for several growth factor receptors, suggesting that suramin might be effective in the treatment of cancer (Betcholtz et al., 1986). However, several investigators have reported on the occurrence of adrenal insufficiency during therapy with this drug (Azerad and Grupper, 1953; Cheson et al., 1986; Stein et al., 1986). We have previously shown that suramin prevents corticotropin (ACTH)-stimulated corticosterone release by dispersed rat adrenocortical cells in vitro (Marzouk et al., 1990). However, it did not prevent cholera toxin-, forskolin- and dibutyryl cyclic AMP (dbcAMP)-stimulated corticosterone release, indicating a selective loss of ACTH stimulatory effects on adrenocortical cells.

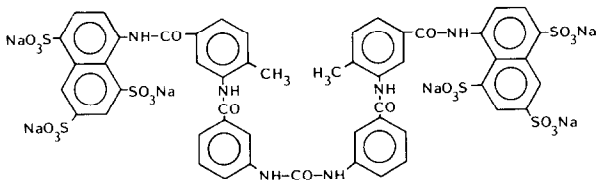

Fig. 1. Structural formula of suramin.

In the present study we evaluated the effect of suramin on basal and secretagogue-altered hormone release by dispersed rat anterior pituitary cells.

Materials and methods

Experimental animals

Female RP rats or male R-Amsterdam rats, weighing 180–200 g, were kept in an artificially illuminated room (08.30–20.30 h) with food and water ad libitum. The animals, in any stage of the estrous cycle, were killed by decapitation between 08.00 and 09.00 h. The pituitary glands were removed within 5 min after killing, the neurointermediate lobe was discarded, and the anterior lobes were collected in calcium- and magnesium-free Hanks' balanced salt solution (HBSS; Gibco, Paisley, U.K.) supplemented with 10 g/l human serum albumin, penicillin (10^5 U/l), streptomycin (100 mg/l), fungizone (0.5 mg/l) and sodium bicarbonate (0.4 g/l).

Pituitary cell culture

The anterior pituitary lobes were dissociated with dispase, a neutral protease from *Bacillus polymyxa* (grade II, final concentration 2.4×10^3 U/l; Boehringer Mannheim, Mannheim, F.R.G.) as described in detail previously (Oosterom et al., 1983a). Viability of the cells, as determined by trypan blue exclusion, was greater than 90%. The dispersed cells were cultured in a culture medium consisting of minimal essential medium with Earle's salts (MEM; Gibco) supplemented with non-essential amino acids, sodium pyruvate (1 mmol/l), 10% fetal calf serum (FCS), penicillin (10^5 U/l), streptomycin (100 mg/l), fungizone (0.5 mg/l), L-glutamine (2 mmol/l) and sodium bicarbonate (2.2 g/l final concentration). The medium was adjusted to pH 7.5 with 1 mol/l NaOH. 10^5 cells per well per 1 ml culture medium were plated in 48-well plates (Coster, Cambridge,

MA, U.S.A.). The studies on ACTH release were carried out after 2 days of culture, since this was previously shown to be the most suitable time of measuring optimal ACTH release by cultured pituitary cells (Lamberts et al., 1986). The studies on prolactin (PRL), growth hormone (GH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release were carried out between days 4 and 7 (Oosterom et al., 1983a; Grootenhuis et al., 1989). The media were refreshed prior to incubation times which were 3 h in the studies on ACTH release, 4 h in the studies on PRL and GH release and 6 or 72 h in the studies on LH and FSH release. No pre-incubations with the investigational compounds were performed. Incubation studies were carried out in MEM only, MEM + 10% FCS or MEM + 0.1% bovine serum albumin (BSA). All studies were done in quadruplicate. At the end of the incubation the media were collected and centrifuged during 5 min at $600 \times g$. The supernatants were stored at $-20\,^{\circ}$C until analysis. The media in which ACTH was determined were adjusted to pH 3.5 using 1.0 N HCl.

Investigational compounds

Ovine corticotropin-releasing factor (oCRF) was obtained from UCB Bioproducts (Brussels, Belgium), vasopressin (VP) from Sandoz (Basle, Switserland), suramin from Bayer (Leverkusen, F.R.G.), growth hormone-releasing hormone (GH-RH) from Universal Biologicals (Cambridge, U.K.), thyrotropin-releasing hormone (TRH) and luteinizing hormone-releasing hormone (LHRH) from Hoechst (Amsterdam, The Netherlands), forskolin from Calbiochem (La Jolla, CA, U.S.A.) and potassium chloride (KCl) from Merck (Darmstadt, F.R.G.). Dopamine (DA), somatostatin (SRIH), dibutyryl cyclic adenosine monophosphate (dbcAMP) and cholera toxin were purchased from Sigma (St. Louis, MA, U.S.A.). Norepinephrine (NE) was supplied by the University Hospital Dijkzigt pharmacy (Rotterdam, The Netherlands). Charcoal-treated bovine ovarian follicular fluid (bFF) was prepared as described previously (Grootenhuis et al., 1989).

Hormone assays

The ACTH concentration in the media was determined by radioimmunosorbent assay (RIA) using a commercially available kit from Medgenix (Fleurus, Belgium). Intra-assay and inter-assay variation amounted to 6.2% and 5.8% respectively. Rat GH and rat PRL concentrations were determined by RIA as described previously (Oosterom et al., 1983a, b). Intra- and inter-assay variations were 8% and 11% for GH and 5% and 8% for PRL respectively. LH and FSH concentrations were determined by RIA as described previously (Grootenhuis et al., 1989), using the antiserum described by Welschen et al. (1975). Intra- and inter-assay variations varied between 10% and 15%. The addition of a sample volume containing 1 mM of suramin had no effect on the initial binding in the RIAs for PRL and LH but it inhibited the initial binding of the RIAs for ACTH, FSH and GH. We overcame interference of suramin in the ACTH and FSH RIAs by diluting the media at least 10 and 5 times respectively. 100 μM and 200 μM suramin had no effect on the initial binding of the RIAs for ACTH and FSH while it still reduced the initial binding in the GH RIA. The suramin present in the media, however, did not affect GH RIA data because the samples were routinely diluted 25 times prior to GH measurement. Each experiment described was repeated at least twice with similar results.

Results are expressed as means $\pm$ SEM and statistical evaluation was done using one-way analysis of variance (ANOVA). Log-transformation of data was used to stabilize variance. For the comparison of differences between means the Newman-Keuls method was applied (Snedecor and Cochran, 1980); $p < 0.05$ was considered significant.

Results

Effects of suramin on ACTH release

First the effect of increasing concentrations of suramin was evaluated on basal and corticotropin-releasing hormone (CRH) (1 nM)-stimulated ACTH release by cultured normal rat anterior pituitary cells (Fig. 2). In cells cultured in MEM + 10% FCS or in MEM + 0.1% BSA neither basal nor CRH-stimulated ACTH release was significantly affected by suramin, even at a concentration of 100 μM. If the cells were cultured in MEM only, however, suramin at concentrations between

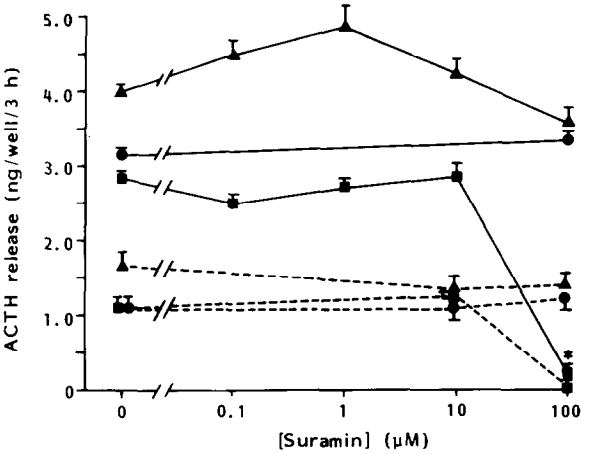

Fig. 2. The effect of increasing concentrations of suramin on basal (- - - - - -) and CRH (1 nM)-stimulated (———) ACTH release by cultured normal rat pituitary cells. Data are mean ± SEM. Culture media were (▲) MEM + 10% FCS, (●) MEM + 0.1% BSA and (■) MEM only. * $p < 0.01$ vs. basal release (without suramin) and vs. CRH only.

0.1 μM and 10 μM did not significantly affect ACTH release, while 100 μM suramin significantly inhibited basal and CRH-stimulated ACTH release ($p < 0.01$ versus respective controls without suramin in both instances).

The mechanism of suramin (100 μM)-mediated inhibition of ACTH release by cells cultured in MEM only was further investigated in the presence of several compounds that stimulate ACTH release by different mechanisms. This is shown in Fig. 3. 100 μM suramin significantly inhibited CRH (1 nM)-, cholera toxin (10 mg/l)-, forskolin (5 μM)-, potassium chloride (56 mM)-, dbcAMP (5 mM)-, vasopressin (VP) (100 U/l)- and norepinephrine (NE) (1 μM)-stimulated ACTH release to a similar extent ($p < 0.01$ in all instances), while 1 and 10 μM suramin had no significant effect.

Effects of suramin on GH and PRL release

The effect of suramin (100 μM) on TRH-stimulated PRL release is shown in Fig. 4. The addition of 100 μM suramin had a synergistic effect on TRH-stimulated PRL release by cells cultured in MEM + 10% FCS (Fig. 4, Table 1; $p < 0.01$ vs. TRH alone at TRH concentrations higher than 0.1 nM), while this effect was not present in cells cultured in MEM + 0.1% BSA (Table 1). Suramin (100 μM) significantly lowered the inhibitory effect of DA (500 nM) on PRL release by cells cultured in MEM + 10% FCS and in MEM + 0.1% BSA (Table 1). 100 μM suramin

TABLE 1

THE EFFECT OF 100 μM SURAMIN ON TRH (100 nM)-STIMULATED AND DA (500 nM)-INHIBITED PRL RELEASE BY RAT ANTERIOR PITUITARY CELLS CULTURED IN MEM + 10% FCS OR IN MEM + 0.1% BSA

Data are mean ± SEM; values in brackets are percentage stimulation (+) or inhibition (−).

Culture medium	Drug (nM)	Suramin (μM)	rPRL (ng/well/4 h)
MEM + 10% FCS	–	–	670 ± 2
MEM + 10% FCS	–	100	820 ± 21 (+22%) [a]
MEM + 10% FCS	TRH (100)	–	1472 ± 38 (+120%) [a]
MEM + 10% FCS	TRH (100)	100	1900 ± 60 (+184%) [a,b]
MEM + 10% FCS	DA (500)	–	280 ± 15 (−58%) [a]
MEM + 10% FCS	DA (500)	100	345 ± 39 (−44%) [a,c]
MEM + 0.1% BSA	–	–	760 ± 16
MEM + 0.1% BSA	–	100	810 ± 44
MEM + 0.1% BSA	TRH (100)	–	1863 ± 59 (+145%) [a]
MEM + 0.1% BSA	TRH (100)	100	2027 ± 97 (±167%) [a]
MEM + 0.1% BSA	DA (500)	–	203 ± 12 (−73%) [a]
MEM + 0.1% BSA	DA (500)	100	353 ± 27 (−54%) [a,c]

[a] $p < 0.01$ vs. control (no drug + no suramin); [b] $p < 0.01$ vs. TRH; [c] $p < 0.01$ vs. DA.

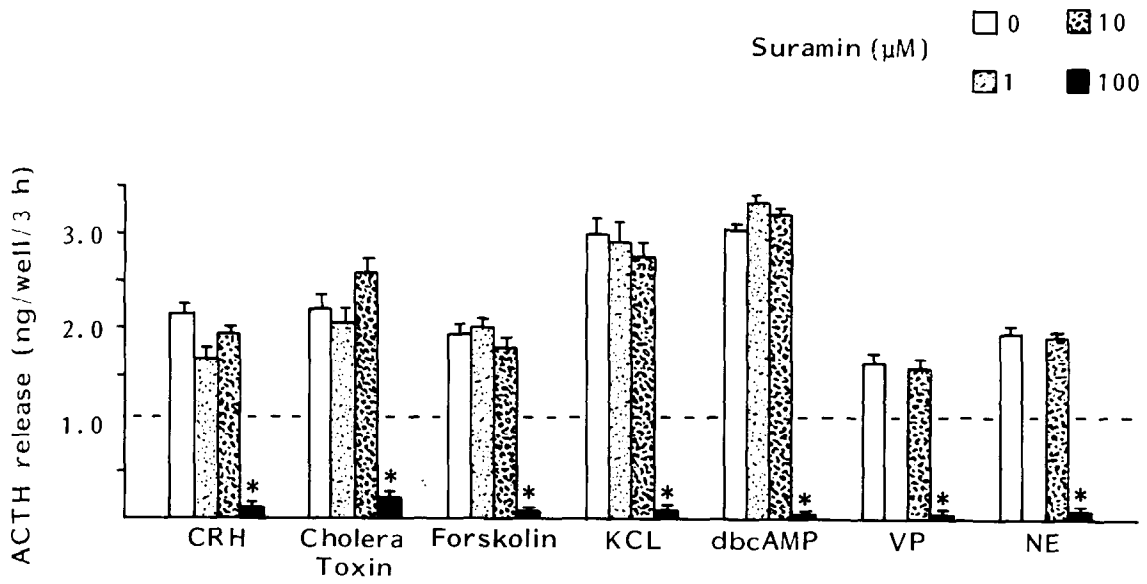

Fig. 3. The effect of suramin on CRH (1 nM)-, cholera toxin (10 mg/l)-, forskolin (5 μM)-, potassium chloride (56 mM)-, dbcAMP (5 mM)-, vasopressin (100 U/l)- and norepinephrine (1 μM)-stimulated ACTH release by normal rat anterior pituitary cells cultured in MEM only. Data are mean ± SEM; the dotted line represents the basal ACTH release. * $p < 0.01$ vs. all the mentioned secretagogues.

had no significant effect on GHRH-stimulated and SRIH-inhibited GH release by cells cultured in MEM + 10% FCS, while it inhibited the GHRH stimulatory effect on GH release by cells cultured in MEM + 0.1% BSA (Table 2). In addition suramin significantly decreased the SRIH inhibi-

TABLE 2

THE EFFECT OF 100 μM SURAMIN ON GHRH (10-nM)-STIMULATED AND SRIH (1 nM)-INHIBITED GH RELEASE BY RAT ANTERIOR PITUITARY CELLS CULTURED IN MEM + 10% FCS OR IN MEM + 0.1% BSA

Data are mean ± SEM; values in brackets are percentage of stimulation (+) or inhibition (−).

Culture medium	Drug (nM)	Suramin (μM)	rGH (ng/well/4 h)
MEM + 10% FCS	–	–	78 ± 4
MEM + 10% FCS		100	84 ± 4
MEM + 10% FCS	GHRH (10)	–	292 ± 17 (+273%) [a]
MEM + 10% FCS	GHRH (10)	100	271 ± 6 (+246%) [a]
MEM + 10% FCS	SRIH (1)	–	48 ± 3 (−39%) [a]
MEM + 10% FCS	SRIH (1)	100	36 ± 4 (−54%) [a,c]
MEM + 0.1% BSA	–	–	137 ± 4
MEM + 0.1% BSA	–	100	130 ± 6
MEM + 0.1% BSA	GHRH (10)	–	540 ± 21 (+293%) [a]
MEM + 0.1% BSA	GHRH (10)	100	351 ± 16 (+156%) [a,b]
MEM + 0.1% BSA	SRIH (1)	–	61 ± 4 (−56%) [a]
MEM + 0.1% BSA	SRIH (1)	100	96 ± 4 (−30%) [a,c]

[a] $p < 0.01$ vs. control (no drug + no suramin); [b] $p < 0.01$ vs. GHRH; and [c] $p < 0.01$ vs. SRIH.

tory effect on GH release by cells cultured in MEM + 0.1% BSA. Finally, in cells cultured in MEM only 100 μM suramin virtually completely suppressed both basal GH and basal PRL release (data not shown).

Effects of suramin on gonadotrophin release

100 μM suramin significantly stimulated the basal FSH release without having a significant effect on basal LH release (Fig. 5; $p < 0.01$ vs. control). On the other hand, 100 μM of suramin caused a decrease of bFF (1.12 nl/ml)-mediated inhibition on FSH and LH release by cells cultured in MEM + 10% FCS (Fig. 5). FSH values increased to 173 ± 12% of those after the addition of bFF alone, whereas LH concentrations increased to 130 ± 7% of the bFF control value. This difference is statistically significant ($p < 0.05$). In order to study the effect of suramin on LHRH-stimulated FSH and LH release we performed 6-h incubation experiments. 100 μM of suramin had no effect on basal or LHRH-stimulated FSH and LH release by cells cultured in MEM + 10% FCS (data not shown).

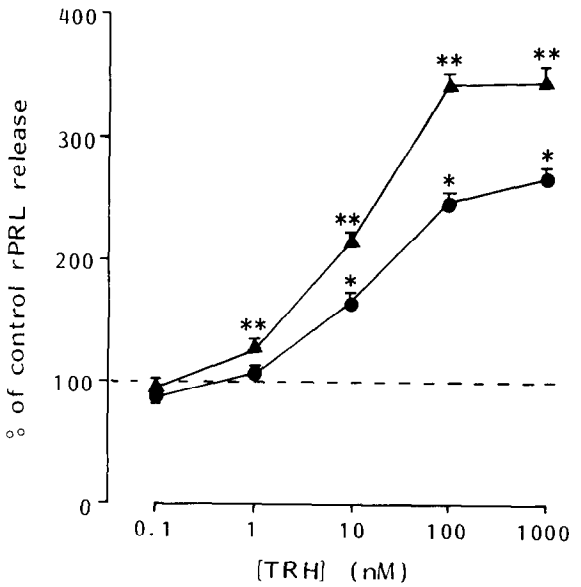

Fig. 4. The effect of 100 μM suramin on TRH-stimulated PRL release by rat anterior pituitary cells cultured in MEM + 10% FCS. Data are mean ± SEM. (●) TRH alone, (▲) TRH + suramin (100 μM). * $p < 0.01$ vs. control; ** $p < 0.01$ vs. TRH alone.

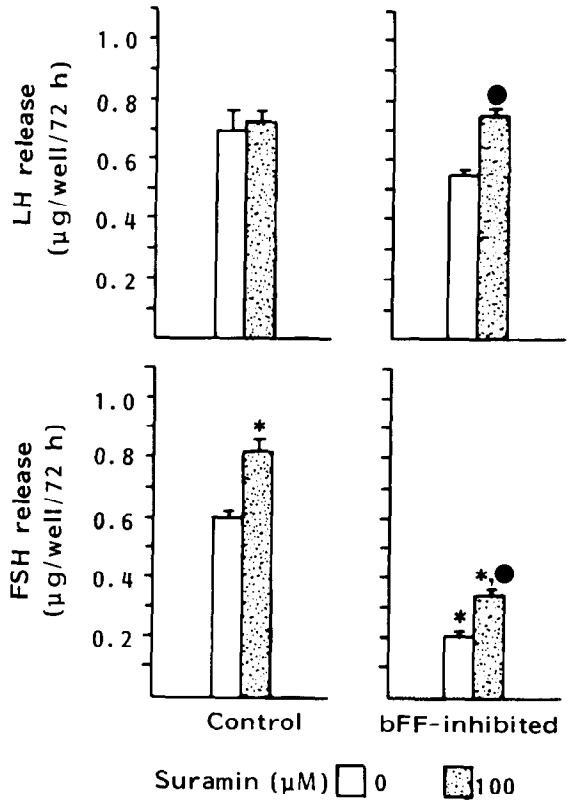

Fig. 5. The effect of suramin on basal and bFF (1.12 nl/ml culture medium)-inhibited LH and FSH release by rat anterior pituitary cells cultured in MEM + 10% FCS. Data are mean ± SEM. * $p < 0.01$ vs. basal LH and FSH release (without suramin); • $p < 0.01$ vs. bFF alone.

Discussion

Suramin, a polyanionic compound, has been demonstrated to inhibit a wide variety of biologic actions in different systems (Hawing, 1978; Mitsuya et al., 1985) via its binding with proteins of all kinds, including albumin, globulins, fibrinogen, histones and a wide variety of enzymes (De Clercq, 1987). Inhibition of glycerol-3-phosphate oxidase and NAD^+-dependent glycerol-3-phosphate dehydrogenase, two key enzymes in glycolysis, explains the antiparasitic action of suramin (Fairlamb and Bowman, 1980).

Pituitary cells probably undergo functional changes when their microenvironments are varied (Martin, 1985). We have investigated the effect of suramin on hormone release by dispersed rat anterior pituitary cells cultured in medium without

serum, and in medium containing 10% FCS or 0.1% BSA. We have used suramin concentrations up to 100 μM because this concentration is near to the therapeutic concentration used in the treatment of AIDS (Stein et al., 1986). Suramin at a concentration of 100 μM inhibited both basal and secretagogue-stimulated ACTH release by dispersed anterior pituitary cells cultured in MEM only, while it had no effect on ACTH release by cells cultured in MEM + 10% FCS or MEM + 0.1% BSA. Basal ACTH release was also significantly inhibited by 100 μM suramin in cells cultured in MEM only. Suramin (10 μM) has been previously reported to inhibit albumin (100 μM)-mediated stimulation of LH-stimulated steroid production by Leydig cells (Bos, 1988). This suggests that in our present study, albumin may interact with suramin and prevent its inhibitory effects on hormone release. This might also mean that the inhibitory effect of suramin (100 μM) on hormone release by pituitary cells cultured in media without protein is non-specific. A toxic effect seems to be the most likely explanation as suramin also inhibited GH and PRL secretion by cells cultured in MEM only. However, if the protein content of culture medium was changed, the effect of suramin (100 μM) on basal and secretagogue-stimulated GH and PRL release greatly varied. Suramin potentiated TRH-stimulated PRL release by pituitary cells cultured in MEM + 10% FCS, while it partially antagonized the inhibitory effect of DA on PRL release by pituitary cells cultured in MEM + 10% FCS as well as in MEM + 0.1% BSA. With respect to GH release suramin inhibited GHRH-stimulated GH release by cells cultured in MEM + 0.1% BSA, while it partially antagonized the SRIH-inhibited GH release by cells cultured in MEM + 0.1% BSA.

Interestingly, 100 μM of suramin stimulated basal FSH release and antagonized the inhibitory effect of bFF on FSH and LH release by pituitary cells cultured in MEM + 10% FCS in 72-h incubation experiments, whereas in 6-h incubation studies the addition of 100 μM suramin had no effect on basal and LHRH-stimulated FSH and LH release. These effects are probably due to the interference of suramin with inhibin, a glycoprotein which inhibits pituitary gonadotrophin production and/or secretion, preferentially that of FSH (Burger, 1988). Inhibin is present in the bFF (De Jong and Sharpe, 1976) and may be produced in the pituitary cells (Roberts et al., 1989). Jansen et al. (1981) have previously reported on the binding of inhibin with Procion red HE3B, a polyanionic dye with a structure similar to that of suramin. Interestingly, preliminary observations showed that Procion red HE3B also inhibited ACTH-stimulated corticosterone release by adrenal cells to a similar extent as that observed with suramin (unpublished observations). These studies suggest that suramin probably binds to the inhibin molecules in the bFF and thus antagonizes its inhibitory effects on FSH and LH release. This hypothesis is substantiated, firstly, because suramin caused suppression of bFF-mediated inhibition of FSH and LH release and secondly, because the effect of suramin is greater on the bFF-mediated inhibition of FSH release than on that of LH release.

It is difficult, however, to envisage why suramin (100 μM) has no effect on CRH-stimulated ACTH release by cells cultured in MEM + 10% FCS and MEM + 0.1% BSA, no effect on LHRH-stimulated FSH and LH release, while it has variable effects on GHRH-stimulated and SRIH-inhibited GH release and TRH-stimulated and DA-inhibited PRL release by these cells. As the properties of amino acid side chains are important for the conformation and function of proteins, for the most part, amino acid with charged, polar or hydrophilic side chains are exposed on the surface of proteins, whereas the non-polar, hydrophobic ones are tucked away inside proteins out of contact with water (Rawn, 1989). The chemical structure of GHRH and SRIH has more basic amino acids which renders them with a strong positive charge while the structures of CRH and LHRH have nearly equal basic and acidic amino acids. The suramin molecules has six negative charges on its surface suggesting a more powerful interaction between suramin and GHRH and SRIH than with the CRH and LHRH molecules. The increase in SRIH-inhibited GH release by 100 μM suramin by cells cultured in MEM + 10% FCS might be explained by interference of suramin with a factor(s) present in the FCS which affects SRIH-inhibited GH release. TRH has no charge, suggesting that the synergistic effect of suramin could be

explained by interaction of suramin with other factor(s) in the FCS which inhibit PRL release. For example, suramin might interact with the PRL molecule itself, intervening its direct negative feedback effect at the level of the lactotroph (Frawley and Clark, 1986).

In summary we suggest that at 100 μM suramin binds to protein molecules in the media with different affinities leading to no effect on CRH-stimulated ACTH release, no effect on LHRH-stimulated FSH and LH release and variable effects on GH and PRL release. The effect of suramin on bFF-mediated inhibition of FSH and LH release, however, seems different. Suramin molecules seem to bind the inhibin preferably rather than to exert non-specific effects on the cultured gonadotrophs.

The fact that suramin combines with a great variety of proteins results, on the one hand, in a lack of specificity, but on the other hand, it also prevents suramin from being eliminated prematurely, permitting the compound to reach sufficiently high blood levels at a dosage regimen of one injection of 1.0 g suramin per week (Collins et al., 1986). Suramin binds for at least 99.7% to plasma proteins, mainly the albumin. As the affinity of suramin binding with albumin is high and the albumin concentration of the human plasma is 3.5–4.5 g/dl, albumin offers probably considerable protection against the inhibitory effects of suramin on pituitary hormone release in vivo.

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