Biochemical Pharmacology, Vol. 36, No. 11, pp. 1825–1828, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

ASSESSMENT OF THE BIOPOTENCY OF ANTI-THYROID DRUGS USING PORCINE THYROID CELLS

S. C. J. READER,* B. CARROLL, W. R. ROBERTSON and A. LAMBERT†
Department of Chemical Pathology, Clinical Sciences Building, Hope Hospital, Eccles Old Road, Salford M6 8HD, U.K.

(*Received* 7 *October* 1986; *accepted* 4 *December* 1986)

Abstract—The effect of six drugs on the uptake and organification of iodide by porcine thyroid cells stimulated with bovine TSH (10 miU/L) has been investigated. The drugs fall into two categories: the peroxidase inhibitors, methimazole (MMI), 2-thiouracil (2-TU) and 3-amino 1,2,4 triazole (3-ATA) and the ionic inhibitors, lithium chloride (LiCl), potassium perchlorate ($KClO_4$), and sodium iodide (NaI). All the drugs led to a dose-related inhibition of iodide metabolism. The most potent effect on iodide uptake was seen with NaI which inhibited this function by 20% even at 10^{-8} mol/l. In contrast, the most potent effect on iodide organification was observed with methimazole which led to a 25% inhibition at 10^{-8} mol/l. The concentrations of drug which gave rise to a 50% inhibition of iodide uptake were (μmol/l) 0.26 (NaI), 3.5 ($KClO_4$), 9.7 (2-TU), 15 (MMI), 26 (3-ATA), and 1500 (LiCl). The comparable figures for organification were 0.13 (MMI), 0.18 (2-TU), 0.23 (NaI), 1.2 (3-ATA), 15 ($KClO_4$) and 1300 (LiCl). We conclude that this *in vitro* system has considerable potential for the assessment of potency and possible bioassay of anti-thyroid drugs of varying structures and sites of action.

There is increasing interest in the development of simple *in vitro* systems which allow investigation into the biological effects of drugs and toxic substances without resource to large scale animal experimentation. To this end, we have recently developed two systems which are suitable for the assessment of the *in vitro* anti-steroidogenic potency of drugs affecting adrenal and testicular steroidogenesis [1, 2]. These methods, which employ isolated guinea-pig adrenal cells and mouse Leydig cells respectively, have proved of value in testing the biopotency of new [3] and existing compounds [4]. We have recently described a porcine cell culture system which is sensitive to 0.1 miU bTSH/l [5] and which is suitable for the detection of thyroid stimulating/inhibiting immunoglobulins [6]. The characteristics of this system have now been exploited to allow assessment of the biological potency of anti-thyroid compounds.

The effect of 6 drugs, methimazole (MMI), 2-thiouracil (2-TU), 3-amino-1,2,4 triazole (3-ATA), lithium chloride (LiCl), potassium perchlorate ($KClO_4$), and sodium iodide (NaI), on iodide uptake and organification by porcine thyroid cells stimulated with 10 miU/l bovine TSH has been investigated.

The thioureylene drugs, methimazole and 2-thiouracil, are used clinically in the treatment of hyperthyroidism and are believed to act at the level of thyroid peroxidase [7], which catalyses the oxidation of iodide and its subsequent incorporation into tyrosine residues of thyroglobulin [8]. The mechanism of action of 3-amino-1,3,4-triazole (3-ATA) is poorly understood but it is believed to be a peroxidase inhibitor.

Uptake of iodide into thyroid cells is dependent upon an energy-dependent pump in the cell membrane. Several inorganic ions, e.g. thiocyanate (SCN^-), nitrate (NO_3^-) and perchlorate (ClO_4^-), have ionic volumes similar to the iodide ion and compete for transport by the iodide carrier. Perchloride, as the potassium salt, is the only ion of the group that finds occasional use in the treatment of hyperthyroidism. Perchlorate is concentrated in the thyroid gland and its action is inhibited by excess iodide which presumably competes for the concentrating mechanism. Perchlorate may produce aplastic anaemia in rare instances and this has severely limited its therapeutic use, and it is now almost obsolete. However, it is occasionally used as a diagnostic aid in thyroid dysfunction.

Although the synthesis of thyroid hormones depends on the presence of iodide, iodide in excess of physiological requirements may inhibit thyroid function. This phenomenon, known as the Wolff–Chaikoff effect, is well described [9, 10]. The response to iodine or iodide in hyperthyroidism is rapid and the maximal effect is achieved within 10–15 days of continuous therapy. The output of hormones is diminished, the gland becomes smaller and firmer and vascularity is reduced. The acute inhibitory effect of excess iodide is a reduction in iodotyrosine and iodothyronine formation. After a variable period the beneficial effect wears off or the condition may become more severe than it was originally. For this reason, the use of iodine and iodide in hyperthyroidism is restricted to preoperative thyroid inhibition prior to thyroidectomy.

Lithium is a small, highly charged cation. It has been used in the treatment of manic depressive psychosis, where a goitrogenic side effect was observed. It has been employed in the treatment of hyperthyroidism [11] but nowadays is rarely used. Due to

* Present address: I.C.I. p.l.c., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ.
† To whom correspondence should be sent.

its polarising nature, it is believed lithium affects ion exchange [11].

We now report on the relative biopotency of these six drugs as inhibitors of iodide uptake and organification by porcine thyroid cells.

MATERIALS AND METHODS

The preparation of primary cultures of porcine thyrocytes has been described in detail elsewhere [5]. Briefly, porcine thyroid glands were obtained from the local abattoir and transported to the laboratory on ice within 1 hr of slaughter. They were chopped into 1 mm^3 pieces under sterile conditions and the tissue pieces were washed twice in Hanks balanced salt solution (Flow Labs, Irvine, Strathclyde) containing penicillin (200,000 units/l), streptomycin (0.2 g/l) and amphotericin B (2.5 mg/l) (medium 1). The tissue was dispersed at 37° in medium 1 containing 0.25% (w/v) Dispase II (Boehringer Mannheim, Lewes, E. Sussex) and 0.05% (w/v) collagenase (grade II, Flow Labs) for 1.25 hr. The cells were filtered and washed three times in Eagle's minimal essential medium (Flow Labs) containing Hepes (20 mmol/l), L-glutamine (2 mmol/l), $NaHCO_3$ (10 mmol/l), Gentamycin (50 mg/l), cortisol (30 μmol/l) and 5% (v/v) heat-inactivated fetal calf serum (medium 2). Finally they were filtered through a 120 μm^2 stainless-steel mesh. Viable cell yields, assessed by trypan blue exclusion, were $>6 \times 10^6$ cells/g fresh tissue and viability was always better than 85%. The cell suspension was diluted to 2×10^6 cells/ml in medium 2 and 500 μl/well were added to 24 well culture plates previously treated with 2 mg/ml poly-L-lysine hydrobromide. The cells were then kept in an incubator at 37° under humidified 5% CO_2:95% air overnight (20 hr).

The effect of 6 drugs, methimazole (MMI), 2-thiouracil (2-TU), 3 amino 1,2,4 triazole (3-ATA), lithium chloride (LiCl), potassium perchlorate ($KClO_4$) and sodium iodide (NaI) on iodide metabolism by the cells was examined. After overnight culture the medium was replaced with HB101 (HANA Media Inc., Berkeley, CA) supplemented with 20 mmol/l Hepes, 1 mmol/l sodium pyruvate, 2 mmol/l L-glutamine, 10 mmol/l sodium bicarbonate, 30 μmol/l hydrocortisone hemisuccinate and 50 mg/l gentamycin sulphate and containing increasing concentrations of the anti-thyroid drugs (10^{-8} to 10^{-2} mol/l) and 10 miu/l bovine TSH. Control wells were also set up with no added drug in the presence and absence of TSH (10 miu/l). This concentration of TSH (10 miU/l) was chosen since both the uptake and organification of iodide by porcine cells is maximally stimulated by this dose [5] (Fig. 1). Incubation at 37° was then continued for 2.5 days.

After this period, radioactive iodide ($Na^{125}I$, 0.2 μCi/well in 3.6 μmol NaI/l) was administered to the cells and the incubation continued for 6 hr [12]. The unlabelled iodide (3.6 μmol/l) was included in order to act as a carrier for the ^{125}labelled iodide and to ensure uniform influx of the radioisotope over the 6 hr incubation period. The medium was removed and the cells washed briefly with ice-cold Hank's balanced salt solution. One ml 100 mmol/l NaOH

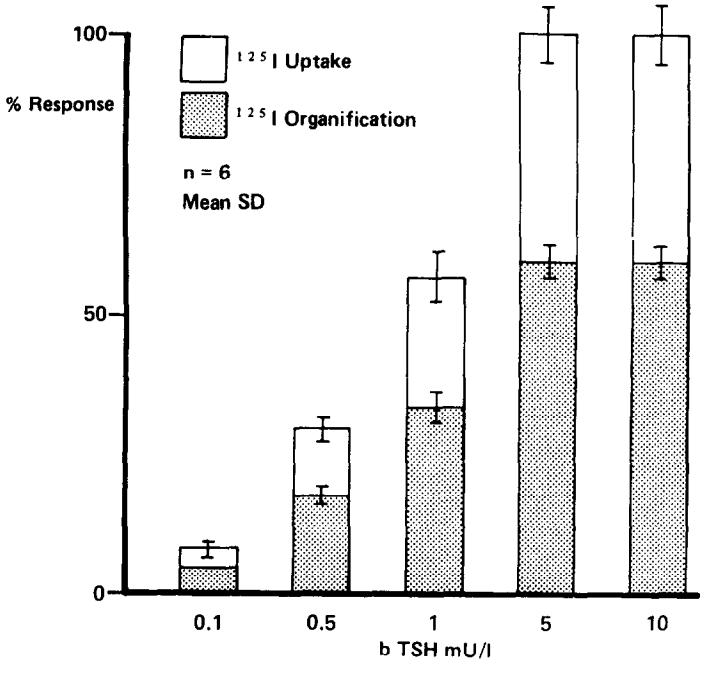

Fig. 1. Percent maximum response of porcine thyroid cells to increasing concentrations of bovine TSH (miU/l). Iodine uptake is represented by the open columns and organification by the hatched columns. Results are presented as mean ± SD (N = 6).

was then added to each well. The cells were solubilized overnight and the total ^{125}iodide in the cell fraction was assessed using a gamma counter (total iodide uptake). The protein bound (organified) ^{125}iodide was precipitated using a final concentration of 40% (w/v) trichloroacetic acid and measured similarly.

Source of chemicals

bTSH (53/11) was obtained from the National Institute for Biological Standards and Control, Holly Hill, London.

Methimazole and 3-amino-1, 2, 4-triazole were purchased from Sigma Chemical Co., Poole, Dorset.

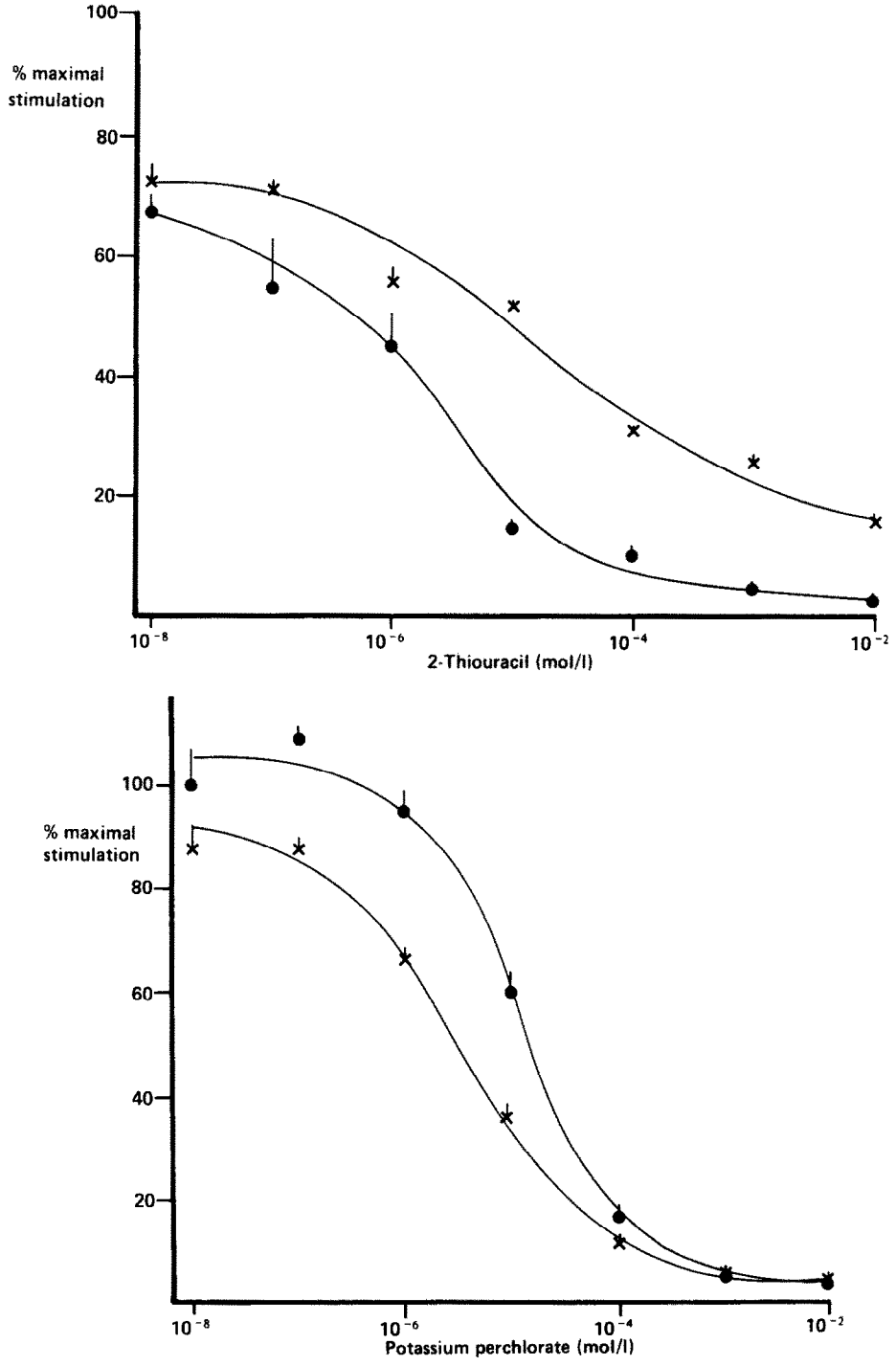

Fig. 2. Effect of increasing concentrations of 2-thiouracil (a) and potassium perchlorate (b) on TSH (10 miU/l)—stimulated iodide uptake (×) and organification (●). The amount of iodide taken up or organified by porcine thyroid cells in response to 10 miU/l in the absence of drugs was taken as the maximal (100%) response. Results are presented as the mean ± SD (N = 4).

Table 1. Concentration of the drugs which led to a 50% inhibition (ED_{50}) of TSH (10 miU/l)-stimulated iodide uptake and organification

Drug	Uptake	ED_{50} (μmol/l) organification	Ratio uptake/organification
NaI	0.26	0.23	1.1
LiCl	1500	1300	1.2
$KClO_4$	3.5	150	0.02
2-TU	9.7	0.18	54
MMI	15	0.13	115
3 ATA	26	1.2	22

The ratio of uptake: organification was calculated as the ratio of the ED_{50} values for uptake and organification respectively.

Lithium chloride and sodium iodide were obtained from B.D.H., Poole, Dorset and potassium perchlorate and 2-thiouracil were purchased from Aldrich Chemical Co., Poole, Dorset.

RESULTS

The biopotency of anti-thyroid drugs

The effect of increasing concentrations of two of the drugs tested, 2-TU and $KClO_4$, on TSH (10 miU/l), stimulated iodide uptake and organification are shown in Fig. 2a,b. The concentration of drug which led to a 50% inhibition (ED_{50}) of iodide uptake and organification is shown in Table 1. All the drugs exhibited a dose related inhibition of iodide metabolism. 2-TU preferentially affected the organification of iodide which is illustrated by its uptake: organification (U:O) ratio of 54, whereas $KClO_4$ preferentially inhibited iodide uptake as demonstrated by its U:O ratio of 0.02 (Table 1). Methiomazole and 3-ATA also preferentially affected the organification of iodide. The most potent effect on iodide organification was seen with methimazole (25% inhibition at 10^{-8} mol/l). Two of the drugs tested, NaI and LiCl, were equipotent as inhibitors of uptake and organification. NaI was the most potent iodide uptake inhibitor tested (20% inhibition at 10^{-8} mol/l) and also highly potent as an inhibitor of organification. LiCl was the least potent inhibitor of both iodide uptake and organification.

DISCUSSION

An *in vivo* system based on cultured porcine thyroid cells and suitable for the estimation of the biopotency of anti-thyroid compounds is described. The assay system is reproducible and technically uncomplicated. This method also gives some indication as to the possible mode of action of the inhibitory substances since two steps in thyroid hormone synthesis, uptake and organification of iodide, may be distinguished.

The anti-thyroid activity of several known goitrogens were examined in this study and all showed an inhibition of iodide metabolism, both at the level of uptake and organification. LiCl was weakly potent as an inhibitor of both iodide uptake and organification. It is possible that lithium acts as a non-specific cytotoxic agent. Of all the drugs studied, only LiCl substantially altered the morphological appearance of the cultured thyrocytes. TSH (10 miU/l) stimulated porcine thyroid cells exhibit a high degree of follicular organization [5]. However, in TSH stimulated cultures in the presence of LiCl at concentrations above 10^{-4} mol/l no follicular-like aggregates were apparent. The highly potent effect of iodide observed in this study would be expected on the basis that iodide, as the natural metabolite of the thyroid gland, must exert sensitive control on thyroid hormone synthesis *in vivo*. Iodide is transported into the cell via membrane proteins which are induced by TSH [13]. *In vitro* the uptake of the ion has been shown to require a long lag phase of up to 24 hr (and presumably relating to protein induction) after the stimulator has been added to the cells [13, 14]. Hence, in order to observe the inhibitory effects of iodide on the uptake of acutely added iodide (over 6 hr), long periods of incubation (2.5 days in this study) with stimulator and inhibitor are required. The recognised modes of action of $KClO_4$ at the level of iodide transport across the plasma membrane [15] and the thiuoureylene drugs, 2-TU and MMI, as inhibitors of thyroid peroxidase [7, 8], a key enzyme involved in iodide organification, are consistent with the results reported here. However, this study also demonstrates that the action of these drugs is not entirely specific in that at higher doses $KClO_4$ also inhibits organification of iodide and 2-TU and MMI inhibit iodide uptake.

We conclude that this simple porcine thyrocyte system has considerable potential for the assessment of the biological potency of anti-thyroid compounds having different structures and modes of action.

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