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Menadione inhibits MIBG uptake in two neuroendocrine cell lines

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Summary

In this paper we report on our studies of the effect of menadione on the uptake of MIBG in the neuroendocrine cell lines PC12 and SK-N-SH. Menadione inhibits the uptake of MIBG in both cell lines in a dose-dependent manner. Inhibition of MIBG uptake is most pronounced in the PC12 cell line. Comparison of the inhibitory action of menadione on the uptake and retention of MIBG with that of imipramine and reserpine suggests that menadione inhibits uptake 1 mediated uptake as well as granular storage.

Introduction

MIBG (*meta*-Iodobenzylguanidine) is a structural analogue of the neurotransmitter norepinephrine. It is recognised by the active norepinephrine transport system, (uptake 1) and accumulates therefore in tissues of neuroendocrine origin [1]. In its radioiodinated form, MIBG is used clinically as a tumour seeking radio pharmaceutical agent for the diagnosis [2] and treatment [3] of neuroendocrine tumours such as neuroblastoma and pheochromocytoma. Long term disease-free survival, especially of stage IV neuroblastoma patients, is yet to be achieved. Therefore, an increase of the effectiveness of the therapy is needed.

The effect of the combination of [^{131}I] MIBG with hyperbaric oxygen is currently studied [4], while other investigators have speculated on the use of a MIBG analogue labelled with astatine-211, [^{211} At] *meta*-astatobenzylguanidine [5], and the use of hyperthermic conditions to improve MIBG uptake [6]. The effect of oxidative stress on the accumulation of MIBG in neuroendocrine cells has not yet been studied, although oxidative stress is known to affect pathways for the accumulation of serotonin, in platelets and catecholamines, in a pheochromocytoma cell line. These pathways are also used for the accumulation of MIBG. Bosin et al. reported increased uptake 1 mediated uptake of serotonin in mice platelets, due to stimulation of the Na^+/K^+ pump ATPase activity by H_2O_2 [7, 8]. Others, however, found a decrease in serotonin uptake in human and mice platelets with increased Cu/Zn Super Oxide Dismutase (Cu/Zn SOD) expression (leading to increased H_2O_2 production) [9], and a decreased uptake of serotonin, norepinephrine and dopamine in the rat pheochromocytoma cell line PC12 transfected with the Cu/Zn SOD gene [10]. The decreased uptake was explained by an impaired granular uptake/storage capacity due to distortion of the ΔpH over the granular membrane. These results suggest, that oxidative stress is potentially capable of improving the accumulation of MIBG in certain neuroendocrine cell types. However, improvement of MIBG accumulation will depend on the granular content of the cell, i.e. the granular contribution to overall cellular uptake.

To demonstrate this, we examined the effect of menadione (a compound that induces oxidative stress when it undergoes redox cycling [11]) on the

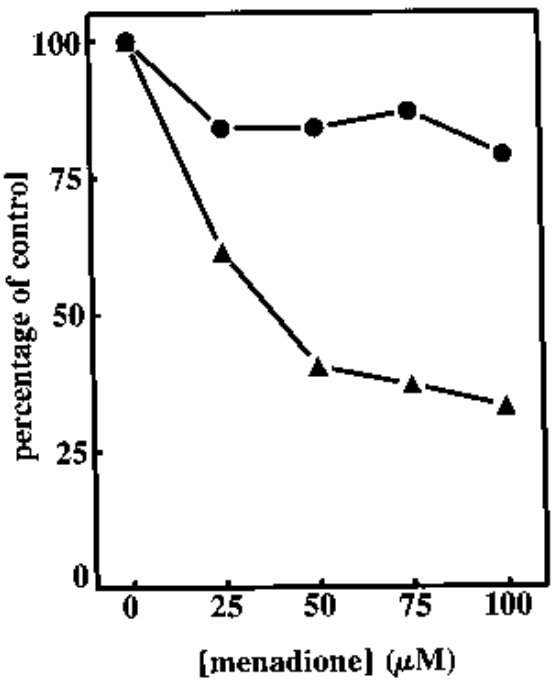

Figure 1. Effect of menadione on uptake of [^{125}I] MIBG in SK-N-SH and PC12 cells. Uptake experiments were performed as described in the section Methods and materials. Results are given as the mean of three independent experiments and represent the percentage of control values (i.e. no menadione added) (13.0 pmol [^{125}I] MIBG/mg protein for the SK-N-SH cells and 9.2 pmol[^{125}I] MIBG/mg protein for the PC12 cells). – ▲ – MIBG uptake in the PC12 cells, – • – MIBG uptake in the SK-N-SH cells.

uptake of MIBG in various neuroendocrine cell lines that are capable of active MIBG uptake. As an example of a cell line with a high granular content, we used the rat pheochromocytoma cell line PC12. The human neuroblastoma cell line SK-N-SH was used as a cell line with a low granular content. In both cell lines we compared the effect of menadione on cellular uptake and retention with that of reserpine (an inhibitor of granular uptake) and imipramine (an uptake 1 inhibitor).

Materials and methods

Cell culture

The human neuroblastoma cell line SK-N-SH, and the rat pheochromocytoma cell line PC12 were cultured at 37° C in a humidified atmosphere of 95% air and 5% CO_2. The SK-N-SH cell line was cultured in DMEM (Bio Whittaker, Verviers, Belgium) supplemented with 10% FCS (Sebak GmbH, Aidenbach, Germany), 10 mM L-glutamine, 100 IU streptomycin and 0.1 mg/ml penicillin (Gibco, Paisley, Scotland). The PC12 cell line was cultured in DMEM/HamF12 (1:1) supplemented with 10% FCS and 10% Horse Serum (Gibco, Paisley, Scotland) and glutamine, streptomycin and penicillin in the concentrations mentioned above. Additionally, for the culturing of PC12 cells, tissue culture flasks were coated with rat tail collagen type I (Sigma Chemical Company, St. Louis, USA) to improve attachment of the cells.

Radiochemicals

[^{125}I]MIBG was prepared by the Cu^{+}-catalysed isotopic exchange method [12], with a specific activity varying from 19.1 to 61.9 mCi/mg.

Uptake experiments

Cells were cultured in 10 cm^2, 6 well culture plates. Culture medium was refreshed after 2 days and menadione (Sigma Chemical Company, St. Louis, USA) was added in the required concentrations. After 10 min, the medium was removed and the cells washed with ice-cold PBS (phosphate buffered saline). Before incubation with [^{125}I] MIBG took place, cells were preincubated for 10 min with 1 ml drug free culture medium under culture conditions. Reserpine (2 μM) and imipramine (10^{-8} M) were added when required, [^{125}I] MIBG was added to a final concentration of 10^{-8} M and the cells were incubated for 5–30 min. Reactions were terminated by removing the medium and by two subsequent washing steps with PBS. [^{125} I] MIBG was extracted from the cells with 0.5 M PCA (perchloric acid) and cellular uptake of [^{125}I] MIBG was determined on a Packard autogamma 5650 gamma counter. The precipitated cells were dissolved in 1 M NaOH and the protein content was determined [13].

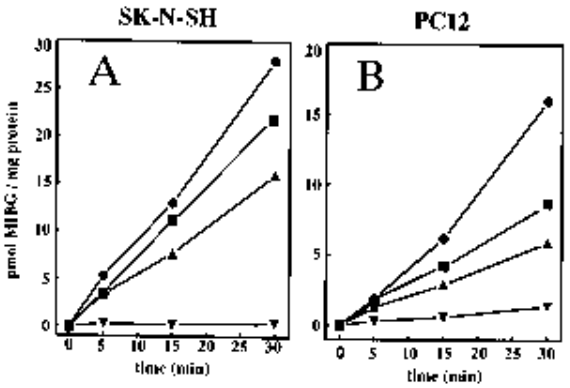

Figure 2. Effect of menadione, imipramine and reserpine on uptake of [^{125}I] MIBG in SK-N-SH and PC12 cells. Uptake experiments in SK-N-SH cells (Fig. 2a) and PC12 cells (Fig. 2b) were performed as described in the section Materials and methods. Results are given as the mean of three independent experiments. – • – control, – ▲ – menadione, – ▼ – imipramine, – ■ – reserpine.

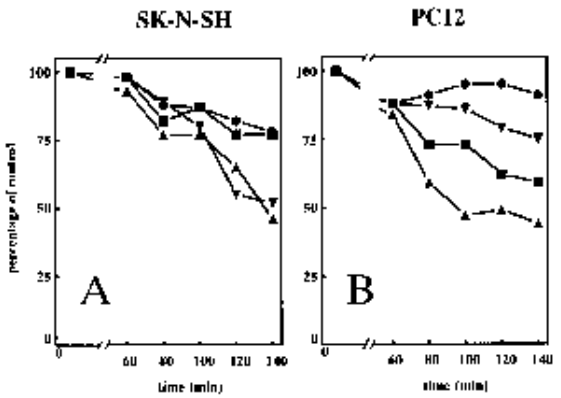

Figure 3. Effect of menadione, imipramine and reserpine on retention of [^{125}I] MIBG in SK-N-SH and PC12 cells. Uptake experiments in SK-N-SH cells (Fig. 2a) and PC12 cells (Fig. 2b) were performed as described in the section Materials and methods. Results are given as the mean of three independent experiments. – • – control, – ▲ – menadione, – ▼ – imipramine, – ■ – reserpine.

Retention experiments

Cells were cultured as described in the 'uptake experiments' section and were incubated with 10^{-8} M [^{125} I] MIBG. After 2 h the incubation medium was replaced by drug free culture medium, the cells remained on this medium for the rest of the experiment. After 1 h on the drug free culture medium, reserpine (2 μM), imipramine (10^{-8} M) or menadione (100 μM) was added. Within certain time intervals after addition of these compounds, cellular [^{125}I] MIBG content was determined, as described in the section 'Uptake experiments'.

Results

In the experiment depicted in Figure 1 the effect of menadione on cellular uptake of MIBG in the PC12 and the SK-N-SH cell line was studied. Both cell lines examined showed a concentration dependent decrease of MIBG uptake after preincubation with menadione. This effect was most pronounced in the PC12 cell line (approximately 70% inhibition at a concentration of 100 μM), the neuroblastoma cell line SK-N-SH was affected to a lesser extent (approximately 25% inhibition at 100 μM).

In order to find out whether the decrease in MIBG uptake was due to inhibition of transport over the cellular membrane (uptake 1) or due to impaired granular storage, we compared the effect of menadione on cellular MIBG uptake and retention, with that of the uptake 1 inhibitor imipramine and the granular storage inhibitor reserpine.

Figure 2 shows the effect of 100 μM menadione, imipramine and reserpine on cellular uptake of MIBG. Imipramine had the strongest effect on cellular uptake of MIBG, virtually no uptake took place in both cell lines. Because of the low granular content of the SK-N-SH cells, reserpine affected MIBG uptake only moderately in these cells (Figure 2a), while the PC12 cells (high granular content) were strongly affected (Figure 2b). The effect of menadione on cellular uptake was stronger than that of reserpine in both cell lines.

Retention experiments (Figure 3) showed a completely different pattern. Reserpine had very little effect on the cellular retention of MIBG in the SK-N-SH cell lines (Figure 3a), but had a profound effect on MIBG retention in the PC12 cells. This resulted in a serious decrease of the cellular MIBG content after 140 min (Figure 3b). Imipramine, however, had a moderately negative effect on MIBG retention in the PC12 cell line but seriously affected MIBG retention in the SK-N-SH cell line. Menadione followed the pattern of imipramine in

the SK-N-SH cell line, whereas, in the PC12 cell line, the negative effect of menadione on MIBG retention exceeded even that of reserpine. This suggests that menadione might have a double effect, on both uptake 1 mediated uptake and on granular storage.

Discussion

This paper presents our studies on the effects of menadione on the overall active uptake of MIBG in two different neuroendocrine cell lines. The neuroblastoma cell line SK-N-SH and the rat pheochromocytoma cell line PC12 were chosen for this study because of their ability to actively accumulate MIBG [14] and because they represent cell lines with low (SK-N-SH) and high (PC12) granular content [15].

Preincubation with increasing concentrations of menadione before incubation with MIBG, led to a dose-dependent decrease of MIBG uptake in both cell lines. This effect of menadione is most likely caused by oxidative stress, which is generated when menadione undergoes redox cycling [11]. Uptake experiments in the presence of H_2O_2 show comparable inhibition of MIBG uptake (data not shown), suggesting that oxidative stress is indeed the cause of the inhibition. Inhibition of MIBG uptake was expected for the PC12 cell line. Oxidative stress is known to partially inhibit the granular storage and thus uptake of serotonin and catecholamines in the PC12 cell line [10] and of serotonin in mice and human platelets [9]. Examining the effect of reserpine (an inhibitor of granular storage) on MIBG uptake in the PC12 cells made it clear that inhibition of granular storage can result in decreased MIBG uptake, this partially explains the decrease in MIBG uptake due to menadione. The negative effect of menadione on the uptake of MIBG, however, is stronger than that of reserpine. The decrease of MIBG uptake in the SK-N-SH cell line due to menadione was surprising because unlike PC12 cells, cells from the SK-N-SH cell line hardly possess granules. Uptake of MIBG is inhibited by menadione nevertheless, suggesting that an additional effect takes place.

Experiments performed by Smets *et al.* [15] show that the PC12 cell line mainly depends on granular storage for cellular retention of MIBG, whereas SK-N-SH cells depend on MIBG re-uptake (i.e. uptake 1). In the retention experiments we performed with SK-N-SH cells, addition of menadione led to a decreased retention capacity of the cells, similar to that of imipramine (an inhibitor of uptake 1). This cannot be due to a reduced granular storage capacity since SK-N-SH cells have very few granules (which is furthermore demonstrated by the lack of effect of reserpine). The PC12 cells' capacity to retain MIBG when imipramine was added was only slightly reduced, whilst reserpine considerably reduced this capacity. Menadione reduced the retention capacity of the PC12 cells for MIBG even stronger. These results suggest that apart from inhibition of granular storage, menadione also inhibits uptake 1 mediated uptake of MIBG.

The negative effect of menadione on uptake and retention of MIBG in the SK-N-SH cells was unexpected. Oxidative stress is known to stimulate the activity of the Na^+/K^+ pump ATPase [8, 16]. This Na^+/K^+ pump ATPase is an important component of the uptake 1 transport system and stimulation of its activity by oxidative stress leads to an increased overall activity of uptake 1 mediated uptake of serotonin in mice platelets [7, 8]. Since the uptake of MIBG into neuronal cells occurs via the same uptake 1 transport system (albeit with the norepinephrine transport protein as transporter [17]), stimulation of the Na^+/K^+ pump ATPase activity in the SK-N-SH cell line was expected to increase MIBG uptake and retention rather than decrease. Bosin *et al.* [8], however, also reported that when the optimal amount of oxidative stress for stimulation of serotonin uptake was exceeded, the uptake decreased again to control values while at the same time the Na^+/K^+ pump ATPase activity remained at its elevated level. They explained this by suggesting that the serotonin transport protein itself was more susceptible to damage by oxidative stress than the Na^+/K^+ pump ATPase. This would lead to a decrease of the activity of the transporter and eventually to the observed normalisation of the overall uptake of serotonin. This theory could also serve as an explanation for the decreased MIBG uptake and retention ability of the SK-N-SH cell line after ex-

posure to menadione in our experiments. If it is indeed the case that addition of menadione leads to a decreased activity of the transport protein used by MIBG, and this negative effect dominates over the positive effect of menadione on the Na^+/K^+ pump ATPase activity, then the overall effect of menadione on the entire uptake 1 transport system will be a negative one. This then explains the negative effect on MIBG uptake and retention in the SK-N-SH cells and the stronger negative effects of menadione (compared to reserpine) on uptake and retention in the PC12 cells.

Independent of the validity of the explanation above can it be concluded that menadione will not be useful in improving the [^{131}I]MIBG accumulation in neuroendocrine tissues. Other ways will therefore have to be found to improve the effectiveness of [^{131}I]MIBG therapy.

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