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Potentiating effect of phenylephrine on isoproterenol activation of thyroxine type II deiodinase in the pineal gland of adult rats

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Abstract. In the present study we show, for the first time, that phenylephrine (PHE), an α-adrenergic receptor agonist, potentiates the effect of isoproterenol (ISO), a β-adrenergic agonist, in activating pineal type II5′-deiodinase (5′-D) activity. The potentiating effect of PHE was observed only at doses of ISO which induce submaximal activation of the enzyme. However, at doses which lead to maximal activation of the enzyme, PHE was ineffective. The results suggest that not only β-, but also α-adrenergic receptors, are involved in the sympathetic noradrenergic regulation of pineal 5′-D activity in the adult rat.
Key words. Pineal; thyroxine; deiodinase; phenylephrine; isoproterenol.

Thyroxine type II5′-deiodinase (5′-D) converts the relatively inactive thyroid hormone T_4 into its highly active metabolite, T_3. This enzyme is present in specific tissues including anterior pituitary, brain, brown adipose tissue, epidermal keratinocytes, the Harderian gland, and the pineal gland[1]. The most important regulatory mechanism for this isoenzyme is the thyroid status. There is a significant increase in its activity during hypothyroidism, and a marked inhibition in the presence of T_4[2,3]. In the rat pineal gland, 5′-D activity is regulated by the light:dark cycle as well as by the thyroid status. The enzyme exhibits a progressive rise in activity after the onset of the dark period and reaches a peak value 5–6 h later; this peak coincides with the peak values described for both melatonin content and N-acetyltransferase (NAT) activity[4–6]. This nocturnal increase in 5′-D activity seems to be dependent on the sympathetic noradrenergic input, since either continuous light exposure or superior cervical ganglionectomy prevents it[5]. Additionally, both in vivo and in vitro studies have shown that isoproterenol (ISO), a β-adrenergic agonist, also activates 5′-D activity, while propranolol, a β-adrenergic blocker, inhibits it[6–10].

In the present study we show for the first time that phenylephrine (PHE), an α-adrenergic agonist, potentiates the effect of ISO, which is known to be a potent activator of pineal 5′-D activity. The results suggest that not only β-, but also α-adrenergic receptors are involved in the sympathetic noradrenergic regulation of pineal 5′-D activity in the adult rat.

Materials and methods

Male Wistar rats, weighing 100–120 g at the time of the study, were allowed to acclimatise to the animal facilities. Animals received food and water ad libitum and were exposed to an automatically regulated light:dark (LD) cycle of 14:10; the lights were turned off daily from 20.00 h to 06.00 h. On the day of the experiment, animals were subcutaneously injected with ISO or/and PHE at 12.00 h and 14.00 h, and killed at 16.00 h. Control animals were injected with saline. Pineals were quickly collected and stored at −80 °C for 5′-D determinations.

The measurement of 5′-D activity was based on the release of radioiodine from [3′,5′-^{125}I]T_4. This method, commonly used in pineal gland deiodinating studies[4–10], is specific for type II isoenzyme because the substrate contains ^{125}I only in the position 5′. Other deiodinating activities, e.g. conversion of T_4 to rT_3, would release only nonradioactive iodide[4]. Pineals were disrupted by ultrasound in 100 µl cold 0.05 M phosphate buffer, pH 6.8. Briefly, 50 µl of a sonicated sample was incubated in the presence of 40 Mm DTT and 2 nM [3′,5′-^{125}I]T_4 as substrate (200 µl final volume). The substrate concentration was similar to the K_m value described for the type II5′-D activity in rat pineal gland[4]. Reaction was started by the addition of substrate and continued for 60 min at 37 °C. Control incubations were performed by omission of the homogenates. The reaction was terminated by the addition of 100 µl cold 2% BSA and 750 µl trichloroacetic acid (10%). Samples were centrifuged for 2 min at 10,000 × g and 500 µl of supernatant was placed on a 0.5 ml column packed with Dowex-50W ion-exchange resin and the column washed twice with 500 µl glacial acetic acid (10%). Radioactivity in the eluate, corresponding to the ^{125}I released, was counted in a gamma counter as an index of 5′-D activity. The recovery of ^{125}I in this process was better than 95%. Specific enzymatic activity was determined by subtracting the control value, which usually amounted to less than 1% of the radioactivity added. 5′-D activity is referred to as ^{125}I released (fmol/gland/h). Results are

expressed as means ± standard errors (SE). Data were statistically analyzed using ANOVA followed by a Student-Newman-Keuls multiple range test.

All reagents were analytical grade and were obtained from commercial sources. T_3, D,L-dithiothreitol (DTT), Dowex-50W, ISO, and PHE were purchased from Sigma (St. Louis, MO, USA); $Na^{125}I$ was purchased from Amersham (Amersham, Bucks, UK). ^{125}I was bound to T_3 using the chloramine T method, as described by Nakamura et al.[11]. [3′,5′-^{125}I]T_4 was purified through a 3-ml Sephadex LH-20 column, and contained less than 2% free iodine.

Results

The effect of increasing doses of ISO and/or PHE on pineal 5′-D activity was studied in adult rats. As shown in figure 1, ISO behaved as a potent activator of the enzyme. ISO slightly increased pineal 5′-D activity at a dose as low as 0.03 mg/kg BW, but exhibited a significant effect at 0.1 mg/kg BW. The maximal effect of ISO was reached at 0.3 mg/kg BW. PHE, however, was incapable of activating the enzyme at any dose studied. The potentiating effect of PHE was observed when PHE and ISO were injected together. At a dose of 0.03 mg/kg BW for both drugs, 5′-D activity was slightly increased above the activity obtained when animals were injected with ISO alone, but when both drugs were injected at doses of 0.1 mg/kg BW, pineal deiodinating activity was clearly enhanced (2-fold above control value with ISO alone). However, the potentiating effect of PHE was observed only at ISO doses which induce submaximal activation of the enzyme (0.1 mg/kg BW). At doses which led to maximal activation of the enzyme by ISO (0.3 or 1.0 mg/kg BW), PHE was ineffective in potentiating the effect of ISO.

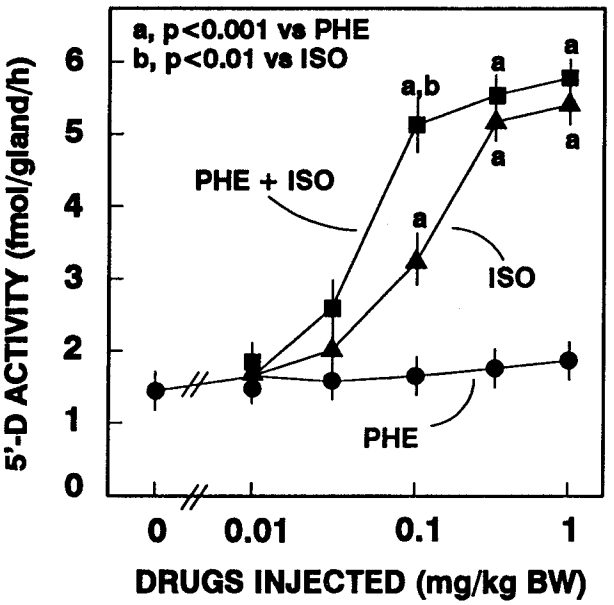

Figure 1. Effect of isoproterenol (ISO), phenylephrine (PHE) or both drugs together (PHE + ISO) on pineal 5′-D activity. Animals were subcutaneously injected with the concentrations indicated of drugs (0, 0.01, 0.03, 0.1, 0.3, and 1 mg/kg/BW) at 12.00 h and 14.00 h, and killed at 16.00 h. Results are expressed as means ± SE of 10 animals.

Discussion

The pineal gland of adult rats is the first tissue in which a potentiating effect of an α-adrenergic receptor agonist, PHE, on 5′-D activity activated by a β-adrenergic agonist, ISO, has been described. Adrenergic regulation of type II5′-D activity has also been studied in other tissues. Silva and Larsen[12] showed for the first time that the deiodinating activity in brown adipose tissue (BAT) was dependent on specific α_1-adrenergic activation of the tissue. In BAT – unlike in the pineal gland – β-adrenergic agonists by themselves had no effect on deiodinating activity, but exerted a synergistic effect on the α-adrenergic stimulation of the enzyme[13]. In the Harderian gland, both α- and β-adrenergic agonists seemed largely to activate the deiodinating activity by themselves[14,15]. Cultured astroglial cells have also been found to contain a type II deiodinating enzyme, which is activated by dibutyryl cyclic AMP, forskolin, ISO, and norepinephrine[16].

On the other hand, the role of β-adrenergic receptors are involved in regulating not only 5′-D activity, but also NAT activity and melatonin production[17]. Thus the physiological role of α-adrenergic receptors remains unclear, because ISO can completely activate pineal NAT[18] and 5′-D[7] activities in adult rats. In these animals, α-adrenergic agonists appear to potentiate the effect of ISO in activating pineal NAT activity[18]. In this report, we also show that PHE can potentiate the effect of ISO in activating pineal 5′-D activity. However, a different type of regulation is present in young rats, where we have also described that PHE by itself can be as potent as ISO in activating pineal 5′-D[19] and NAT[20,21] activities. Therefore, α-adrenergic receptors may be mainly involved in the regulation of pineal function in the growing rat, whereas β-adrenergic receptors play a role in adult rats.

In conclusion, activation of pineal 5′-D activity by ISO is potentiated by PHE, although the physiological significance of this effect remains unclear. Further studies are required for a better understanding of the physiological role of α-adrenergic receptors in regulating pineal 5′-D activity in adult rats. However, this and previous results in young rats suggest that α-adrenergic receptors should be included among the physiological regulators of pineal deiodinating activity.

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1 Guerrero, J. M., and Reiter, R. J., Int. J. Biochem. *24* (1992) 1513.
2 Dratman, M. B., Crutchfield, F. L., Gordon, J. T., and Jennings, A. S., Am. J. Physiol. *245* (1983) E184.
3 Silva, J. E., and Leonard, J. L., Endocrinology *116* (1985) 1627.
4 Tanaka, K., Murakami, M., and Greer, M. A., Biochem. biophys. Res. Commun. *137* (1986) 863.
5 Guerrero, J. M., Puig-Domingo, M., and Reiter, R. J., Endocrinology *122* (1988) 236.
6 Guerrero, J. M., Puig-Domingo, M., Santana, C., Menendez-Pelaez, A., and Reiter, R. J., J. pineal Res. *5* (1988) 513.
7 Guerrero, J. M., Puig-Domingo, M., Santana, C., Menendez-Pelaez, A., and Reiter, R. J., Cell. molec. Neurobiol. *8* (1988) 447.
8 Guerrero, J. M., Santana, C., and Reiter, R. J., Neurosci. Lett. *89* (1988) 229.
9 Murakami, M., Greer, S. E., McAdams, S., and Greer, M. A., Neuroendocrinology *50* (1989) 88.
10 Guerrero, J. M., Santana, C., and Reiter, R. J., Proc. Soc. exp. Biol. Med. *194* (1990) 327.
11 Nakamura, Y., Chopra, I. J., and Solomon, D. H., J. nucl. Med. *18* (1977) 1112.
12 Silva, J. E., and Larsen, P. R., Nature *305* (1983) 712.
13 Raasmaja, A., and Larsen, P. R., Endocrinology *125* (1989) 2502.
14 Osuna, C., Rubio, A., Goberna, R., and Guerrero, J. M., Life Sci. *46* (1990) 1951.
15 Rubio, A., Guerrero, J. M., Gonzalez, M. A., Lopez-Gonzalez, M. A., and Osuna, C., Life Sci. *49* (1991) 1523.
16 Courtin, F., Chantoux, F., Pierre, M., and Francon, J., Endocrinology *123* (1988) 1577.
17 Reiter, R. J., Endocr. Rev. *12* (1991) 151.
18 Klein, D. C., Sudgen, D., and Weller, J. L., Proc. natl Acad. Sci. USA *80* (1983) 599.
19 Rubio, A., Osuna, C., and Guerrero, J. M., Endocrinology *128* (1991) 1661.
20 Rubio, A., Osuna, C., Jimenez, J., Molinero, P., and Guerrero, J. M., Neurosci. Lett. *127* (1991) 13.
21 Rubio, A., Guerrero, J. M., Reiter, R. J., and Osuna, C., Endocrinology *132* (1993) 393.