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Isoprenaline-induced changes in activity of the endogenous inhibitor of cAMP-dependent protein kinases under conditions of β-adrenoceptor supersensitivity

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Isoprenaline-induced changes in activity of an endogenous, specific inhibitor of cAMP-dependent protein kinases (type I inhibitor) under conditions of experimental supersensitivity of β-adrenoceptors were investigated in rat hippocampus and brain stem. Both subchronic administration of reserpine (2·5 mg kg^{-1}, i.p., 4 days, once daily) and i.c.v injections of 6-hydroxydopamine (250 μg/ventricle, bilaterally, 48 h apart), which are known to increase β-adrenoceptor sensitivity in the rat brain, markedly enhanced the response of the type I inhibitor activity to isoprenaline. To obtain a significant decrease of type I inhibitor activity in the examined brain structures of these animals, doses of isoprenaline 2–5 times lower than in control groups had to be used. It is suggested that the isoprenaline-induced decrease of type I inhibitor activity might be used as an index of central β-adrenoceptor reactivity in-vivo.

An endogenous, small molecular weight and thermostable protein that inhibits cAMP-dependent protein kinases (a type I inhibitor) is widely distributed among tissues. The type I inhibitor selectively binds to free catalytic subunits of cAMP-dependent protein kinases, giving an inactive complex, and in this way blocks the enzymes, activity (Ashby & Walsh 1972; Demaille et al 1977). It has been suggested that the type I inhibitor may function in regulating protein phosphorylation mediated by a cAMP-dependent system (Walsh 1978). Stimulation of adenylate cyclase-coupled β-adrenoceptors or dopamine receptors induces an increase of cAMP content followed by liberation of free, active catalytic subunits of cAMP-dependent protein kinase. Simultaneously, a decrease of the type I inhibitor activity is observed (Szmigielski 1981, 1984). Recently we have found that the subsensitivity of β-adrenoceptors in rat brain, produced by long-term treatment with either some antidepressant drugs or electroconvulsive shocks, is accompanied by the reduced responsiveness of the type I inhibitor to the isoprenaline-induced stimulation of these receptors (Szmigielski et al 1984). Several lines of evidence (binding studies, increased responsiveness of the brain noradrenaline=sensitive cAMP generating system) indicate that in-vivo deprivation of noradrenaline at postsynaptic sites caused by either reserpine or intraventricularly given 6-OHDA, resulted in a supersensitivity of β-adrenoceptors in rat brain (Sporn et al 1977; U'Prichard & Snyder 1978; Vetulani et al 1976).

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It was the aim of this study to extend previous findings on the characterization of the responsiveness of the type I inhibitor to isoprenaline and to assess how supersensitivity of central β-adrenoceptors, produced by reserpine or 6-OHDA, affects this responsiveness.

Methods

Male, albino Wistar rats, 160–200 g, were kept at 20–22 °C, under 12 h dark-light cycle, housed ten to a cage, with free access to granulated food and tap water. Reserpine (Ciba) was administered i.p., once daily, for 4 days in a dose of 2·5 mg kg^{-1}. Control animals received 1 ml kg^{-1} of 0·9% NaCl (saline). The rats were decapitated 24 h after the last injection. 6-Hydroxydopamine hydrobromide (6-OHDA; Sigma) was dissolved in ice-cold saline containing 1 mg ml^{-1} of ascorbic acid immediately before use and was injected i.c.v. in a volume of 10 μl, twice in a dose of 250 μg (48 h apart). Control rats received i.c.v. 10 μl of saline containing 1 mg ml^{-1} of ascorbic acid. The animals were decapitated 21 days after the first i.c.v. injection. The animals from all the tested groups were injected i.p. with saline or various doses of isoprenaline (Germed) 10 min before death. In some experiments rats received i.p. propranolol (Polfa) or aminophylline (Sigma), 20 or 30 min before death, respectively. The type I inhibitor activity was measured in hippocampus and brain stem, as described elsewhere (Szmigielski 1981; Szmigielski et al 1984). One unit of the type I inhibitor activity was defined as the amount of this inhibitor which at 30 °C blocks transfer of phosphate (catalysed by cAMP-dependent protein kinase) from [^{32}P]ATP into histone by 4 pmol min^{-1}.

Student's *t*-test was used for statistical analysis of the results.

Results

Isoprenaline produced a dose-dependent decrease of the type I inhibitor activity in rat hippocampus and brain stem. Statistically significant reduction in the type I inhibitor activity was observed after a dose of 1 mg kg^{-1} of the drug (Fig. 1).

Subchronic treatment with reserpine markedly enhanced the response of the type I inhibitor activity to isoprenaline in the rat hippocampus and brain stem. Isoprenaline in a dose of 0·2 mg kg^{-1} (which practically

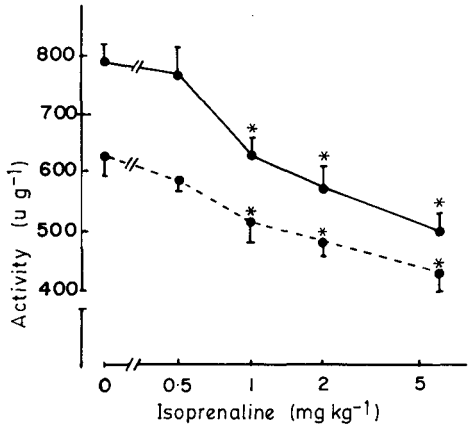

FIG. 1. The effect of various doses of isoprenaline on the type I inhibitor activity in hippocampus and brain stem of normal rats. Each point represents the mean ± s.e.m. from 5–6 animals *$P < 0.05$ when compared to the saline treated group. ●——● hippocampus, ●- - -● brain stem.

was without effect in the control group) produced a significant decrease of the type I inhibitor activity in these two brain structures. The basal type I inhibitor activity was similar in non-reserpinized and reserpinized rats (Fig. 2A, B). In the hippocampus of the reserpinized animals the studied isoprenaline action was blocked by propranolol and enhanced by aminophylline (Table 1).

Chemical degeneration of central catecholaminergic neurons with 6-OHDA increased the responsiveness of the type I inhibitor to isoprenaline. Both in the hippocampus and brain stem of the rats treated with 6-OHDA a statistically significant decrease of the type I inhibitor activity was observed after a dose of isoprenaline two times lower than in the control animals. 6-OHDA did not change the basal type I inhibitor activity in the rat brain structures (Fig. 3A,B).

Table 1. The effect of propranolol and aminophylline on the isoprenaline-induced decrease of the type I inhibitor activity in hippocampus of reserpinized rats.

Treatment	Type I inh. activity (u g^{-1})
Saline	790 ± 20
Isoprenaline 0·1 mg kg^{-1}	710 ± 35
Isoprenaline 0·5 mg kg^{-1}	540 ± 41[a]
Aminophylline 60 mg kg^{-1}	730 ± 15
Aminophylline 60 mg kg^{-1} + Isoprenaline 0·1 mg kg^{-1}	590 ± 25[b]
Propranolol 5 mg kg^{-1}	800 ± 25
Propranolol 5 mg kg^{-1} + Isoprenaline 0·5 mg kg^{-1}	765 ± 20[b]

Each value represents the mean ± s.e.m. from 5–6 animals.
[a] $P < 0.05$ when compared to the saline-treated group.
[b] $P < 0.05$ when compared to the isoprenaline-treated group.

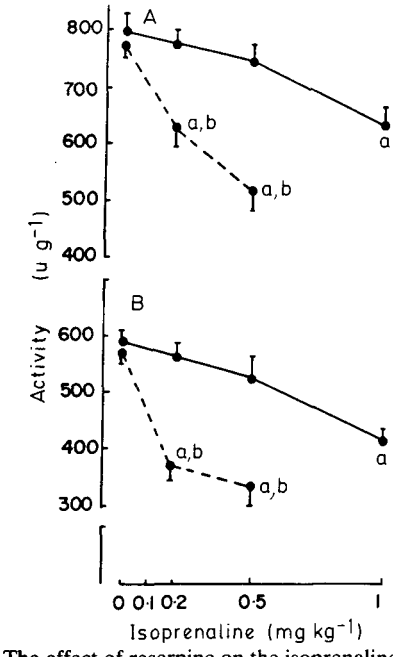

FIG. 2. The effect of reserpine on the isoprenaline-induced decrease of the type I inhibitor activity in rat hippocampus (A) and brain stem (B). Each point represents the mean ± s.e.m. from 5–6 animals. a, $P < 0.05$ when compared to the saline-treated group; b, $P < 0.05$ when compared to the group which received the same dose of isoprenaline. ●——● control, ●- - -● reserpine.

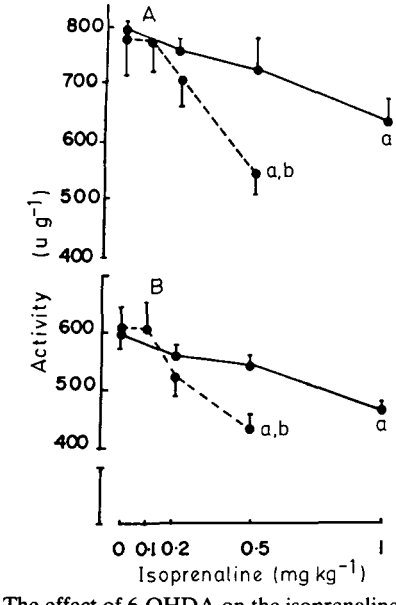

FIG. 3. The effect of 6-OHDA on the isoprenaline-induced decrease of the type I inhibitor activity in rat hippocampus (A) and brain stem (B). Each point represents the mean ± s.e.m. from 5–7 animals. a, $P < 0.05$ when compared to the saline-treated group; b, $P < 0.05$ when compared to the control group which received the same dose of isoprenaline. ●——● control, ●- - -● 6-OHDA.

Discussion

Systemic administration of isoprenaline results in a rapid and short-lasting increase of cAMP content in the mouse brain (Westerman 1973). Moreover, Frances & Simon (1978) reported hypomotility in mice 30 min after i.p. injection of isoprenaline; a behavioural phenomenon was due to the stimulation of central β-adrenoceptors. It has been shown that isoprenaline produced a dose-dependent decrease of type I inhibitor activity in rat hippocampus and brain stem, the effect being related to both the stimulation of β-adrenoceptors and an increase in cAMP content (Szmigielski et al 1984; present results). Isoprenaline-induced decrease of the type I inhibitor activity in the rat brain was observed up to 30 min after the i.p. administration of the drug (data not shown).

Earlier, we proposed that changes in the sensitivity of β-adrenoceptors in rat brain in-vivo affected the responsiveness of the type I inhibitor to isoprenaline. Thus, the subsensitivity of these receptors produced by repeated administration of some antidepressant drugs (i.e. imipramine, nomifensine and mianserin), as well as by a series of 11 electroconvulsive shocks, was followed by the diminished response of type I inhibitor activity to the isoprenaline action. In animals subjected to these antidepressant treatments a significant decrease in the type I inhibitor activity was observed after a five times higher dose of isoprenaline than in control rats. In contrast, acute treatment with either the drug or electroconvulsive shock had no influence on the isoprenaline-induced decreases of the type I inhibitor activity (Szmigielski et al 1984). The present results give further support for the concept mentioned above. We have found that after subchronic treatment of reserpine or i.c.v. injections with 6-OHDA, much lower doses of isoprenaline than in the control animals produced a significant reduction of the type I inhibitor activity in rat hippocampus and brain stem. Blockade of the isoprenaline action by propranolol, but not by phentolamine or haloperidol (results not shown), and enhancement by aminophylline (an inhibitor of phosphodiesterase) might indicate the involvement of changes in the β-adrenoceptor sensitivity in the observed phenomenon. It has been reported by several authors that reserpine-induced depletion of central catecholamines or 6-OHDA-induced destruction of central catecholaminergic neurons caused the supersensitivity of β-adrenoceptors in rat brain (Vetulani et al 1976; Sporn et al 1977; U'Prichard & Snyder 1978). Therefore, the findings presented in this paper suggest that the increased sensitivity of these receptors is accompanied by the enhancement of the responsiveness of the type I inhibitor to isoprenaline.

From our results we suggest that the determination of the response of the type I inhibitor activity to isoprenaline may be used as a biochemical test to evaluate central β-adrenoceptor sensitivity in-vivo.

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