J. Pharm. Pharmacol. 1983, 35: 677–679
Communicated January 5, 1983

Effect of withdrawal from chronic propranolol treatment on high-affinity choline uptake in rat brain

L. CHARLES MURRIN*, RICHARD H. KENNEDY†, THOMAS E. DONNELLY, JR, *Department of Pharmacology, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, NE 68104, U.S.A.*

Several laboratories have reported the distribution and properties of noradrenergic β-adrenoceptors in rat brain (Alexander et al 1975; Bylund & Snyder 1976; Minneman et al 1979). The function of these receptors and their interaction with other neuronal systems is not well understood particularly in the striatum in which there is a high density of β-adrenoceptors yet a sparse noradrenergic innervation. We have examined the effects of chronic treatment with (±)-propranolol and subsequent withdrawal for possible effects on the cholinergic neurons in the striatum by measuring high-affinity choline uptake. Parts of the data have been briefly reported (Murrin et al 1980).

Methods and materials

Ten week old male albino rats (Wistar Kyoto rats or, where noted, spontaneously hypertensive Okamoto-Aoki strain (SHR) Rats, Taconic Farms, Taconic, N.Y.) were given intraperitoneal injections of 10 ml kg^{-1} (±)-propranolol (Ayerst Labs, New York, N.Y.) dissolved in sterile 0·9% NaCl (saline) three times daily (30 mg kg^{-1} total per day) for 16 days. The propranolol was prepared fresh daily. Controls received injections of saline on the same schedule and were littermates of treated animals. Propranolol treatment had no effect on weight gain during the experiments. Animals withdrawn from propranolol were killed 16 h after the last injection while those not withdrawn were killed 2 h after the last injection. Animals treated with reserpine were given 5 mg kg^{-1} i.p. once a day for five days and killed 24 h after the last injection.

High-affinity choline uptake was measured as previously described (Murrin & Kuhar 1976). Briefly, brain regions were homogenized in 20 vol of ice-cold 0·32 M sucrose and a synaptosomal preparation (P_2, crude mitochondrial prep) was prepared by differential centrifugation. The pellets were resuspended in 20 vol of 0·32 M sucrose. Uptake studies were carried out in Krebs-Ringer Phosphate buffer (KRP mM; NaCl 122, KCl 4·9, $CaCl_2$ 1·6, $MgCl_2$ 1·2, Na_2HPO_4 15·8, dextrose 11), pH 7·4, 37 °C. Synaptosomes (0·1 ml) were incubated in 0·9 ml KRP for 5 min at 37 °C. [^{3}H]Choline (Amersham, Chicago, IL; 10 Ci $mmol^{-1}$) was then added to a final concentration of 50 nM, and the incubation continued for 4 min. The experiments were stopped by adding 2 ml of ice-cold KRP followed by centrifugation. Blanks were determined by using zero-sodium KRP in which sucrose substituted for NaCl. Pellets from the assays were dissolved in Protosol (New England Nuclear, Boston, MA) and counted by liquid scintillation spectrophotometry. Triplicate assays of each tissue were used throughout and typically had a range of less than 10%. Blanks were also carried out in triplicate and accounted for as little as 5–10% of total uptake in striatum to as much as 40–50% of total uptake in hypothalamus. Statistical analyses used Student's paired *t*-test. Animals were paired for analysis on the basis of being littermates, of being treated on the same days, and of being assayed at the same time. This corrected for variations in the assays which were carried out over a period of several months (propranolol-withdrawal; Table 1).

Results

In animals treated with (±)-propranolol for 16 days followed by a 16 h withdrawal, there was a significant (34%) increase in high-affinity choline uptake in the

Table 1. High affinity choline uptake in brain regions after propranolol treatment. Animals were treated with (±)-propranolol (30 mg kg^{-1} day^{-1}) for 16 days and withdrawn from the drug 16 h before death. See Methods for details. Data are mean ± s.e.m. n = number of matched pairs. Variability among the assays is due to the fact that they were carried out at various times over three months.

Region	Choline uptake (Na-dep pmol/4 min g-mg prot) Control	Treated	n
Cortex	4·45 ± 0·51	4·17 ± 0·36	10
Hippocampus	5·45 ± 0·48	5·33 ± 0·48	10
Hypothalamus	4·10 ± 0·58	3·43 ± 0·41	9
Striatum	13·64 ± 1·83	18·27 ± 2·79*	9
Striatum (SHR)†	17·30 ± 2·06	21·86 ± 2·06*	5

* Significantly different from control, $P < 0·05$. (For d = difference in response for each matched pair, Σd and Σd², respectively for each region are: cortex −3·23, 9·22; hippocampus −1·20, 3·72: Hypothalamus −6·38, 23·7; striatum 41·7, 441; striatum (SHR) 22·8, 136.)

† Data from spontaneously hypertensive Okamoto-Aoki rats.

* Correspondence.
† Present address: Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI, U.S.A.

striatum (Table 1). This increase in choline transport was seen only in the striatum and not in any of the other brain regions that were examined. In addition, this effect was also seen in the striatum of spontaneously hypertensive Okamoto-Aoki strain rats, which normally have much higher basal levels of high-affinity choline uptake compared with normotensive rats [Striatum (SHR); Table 1]. As in the normotensive rats, no effect on choline uptake was seen in other brain regions.

Rats killed 2 h after the last dose of propranolol therefore did not go through a withdrawal period, high-affinity choline uptake was not significantly different from control animals in any brain region studied (data not shown).

In contrast to the above experiments, treatment of rats with reserpine for five days led to significant increases in the rate of high-affinity choline transport in cortex and hippocampus as well as striatum (Table 2).

To examine the possible involvement of β-adrenoceptor supersensitivity in this effect, animals that had been withdrawn from propranolol for 15·5 h were then given (±)-isoprenaline, a β-adrenoceptor agonist, at a dose of 10 ml kg^{-1} i.p., and killed 30 min later (16 h withdrawal from proporanolol). This produced an even greater increase in striatal high-affinity choline uptake. Animals withdrawn from (±)-propranolol for 16 h had sodium-dependent choline uptake levels of 17·8 ± 1·9 pmol/4 min-mg protein, similar to the effect seen in Table 1. Those animals withdrawn from (±)-propranolol for 16 h and treated with (±)-isoprenaline (10 ml kg^{-1} i.p.) 30 min before death had sodium-dependent choline uptake levels of 21·1 ± 2·0 pmol/4 min-mg protein. This was significantly different from values in animals withdrawn from (±)-propranolol but not treated with (±)-isoprenaline ($P < 0·05$, n = 3 pairs). Treatment of rats with (±)-isoprenaline without previous chronic (±)-propranolol treatment produced no significant effect on choline uptake in the striatum compared with controls. This indicates that supersensitive β-adrenoceptors were involved in the changes in high-affinity choline uptake.

Discussion

The striatum has one of the highest regional concentrations of β-adrenoceptors and β-adrenoceptor-sensitive adenylate cyclase (Alexander et al 1975; Minneman et al 1979) but a sparse noradrenergic innervation (Brownstein et al 1974). While acute treatment with propranolol does not appear to affect the function of dopaminergic neurons in the striatum (Fuxe et al 1976; Wiesel 1976; Morinan & Leonard 1977), chronic treatment produces an increase in striatal DA concentrations and so apparently affects dopaminergic function (Morinan & Leonard 1977).

We have examined whether chronic propranolol treatment also affected cholinergic function in the c.n.s. as indicated by changes in the rate of high-affinity choline uptake. High-affinity choline uptake in synaptosomes has been shown to be a sensitive and useful marker for the level of cholinergic neuronal activity (Atweh et al 1975; Kuhar & Murrin 1978; Simon & Kuhar 1975). The results suggest that β-adrenoceptors in the striatum do affect cholinergic function. We found that chronic propranolol treatment followed by a 16 h withdrawal led to an increase in high affinity choline uptake. This strongly suggests an increase in cholinergic neuronal activity (Atweh et al 1975). Among the brain regions studied the effect was found only in the striatum. This is in contrast to the results with reserpine, which affects many monoamine systems via the same general mechanism as propranolol, i.e. blockade of neurotransmission at the synapse while the specific effect of reserpine is different, being depletion of monoamine stores. The increase of choline uptake in the striatum following reserpine is probably due to depletion of dopamine and subsequent loss of an inhibitory input to the cholinergic interneurons. This suggests stimulation of dopaminergic and β-adrenoceptors has opposite effects on cholinergic systems in the striatum, the former being inhibitory and the latter excitatory. The fact that the propranolol effect was localized to one region indicates it is not a general property of β-adrenoceptor blockade and subsequent supersensitivity.

The effect of propranolol may be a hypersensitivity response due to a supersensitivity of β-adrenoceptors in the striatum. This is supported by the data showing an isoprenaline stimulus produces a further increase in choline uptake in propranolol-treated animals. The need for a withdrawal period before the supersensitivity effect can be seen probably is due to the necessity of washing out the endogenous propranolol from the striatum.

The fact that withdrawal from chronic propranolol alters choline uptake in the striatum is all the more interesting in light of the fact that the choline uptake system in the striatum appears to be less responsive to

Table 2. High affinity choline uptake in brain regions following reserpine treatment. Animals were treated with reserpine (5 mg kg^{-1} day^{-1}) for five days before the experiments then killed 24 h after the last injection. See Methods for details. Data are mean ± s.e.m. n = number of matched pairs.

Region	Choline Uptake (Na-dep pmol/4 min-mg prot) Control	Treated	n
Cortex	5·18 ± 0·55	6·12 ± 0·48*	10
Hippocampus	6·57 ± 0·54	7·30 ± 0·57*	11
Hypothalamus	5·21 ± 0·55	4·26 ± 0·24	9
Striatum	15·64 ± 1·63	22·63 ± 2·59*	8

* Significantly different from control, $P < 0·05$. (Σd, Σ^2 respectively: cortex 9·42, 22·0; hippocampus 8·02, 12·9; hypothalamus −8·52, 39·6; striatum 55·9, 763; (see Table 1 footnote for further details.)

increased depolarization of cholinergic terminals than in other brain regions (Sherman et al 1978; Murrin & Kuhar, unpublished results). This suggests that the effects of propranolol are particularly strong.

The localization of β-adrenoceptors in the striatum and how they modulate cholinergic function is not clear. It has been shown that they do not diminish after kainic acid lesions (Zahniser et al 1979), suggesting that the receptors are not on the cholinergic neurons themselves. Thus β-adrenoceptor stimulation may act indirectly on cholinergic neurons, perhaps by altering the release of another neurotransmitter. While a decrease in dopamine release, as suggested by the data of Morinan & Leonard (1977), would be consistent with an increase in cholinergic activity, there is recent evidence which indicates this may not be the mechanism of propranolol's action (Reisine et al 1982). At this time the dominant site for propranolol's effect on cholinergic neurons remains to be established.

REFERENCES

Alexander, R. W., Davis, J. N., Lefkowitz, R. J. (1975) Nature (London) 258: 437–440

Atweh, S., Simon, J. R., Kuhar, M. J. (1975) Life Sci. 17: 1535–1544

Brownstein, M., Saavedra, J. M., Palkovits, M. (1974) Brain Res. 79: 431–436

Bylund, D. B., Snyder, S. H. (1976) Mol. Pharmacol. 12: 568–580

Fuxe, K., Bolme, P., Agnati, L., Everitt, B. J. (1976) Nuerosci. Lett. 3: 45–52

Kuhar, M. J., Murrin, L. C. (1978) J. Nuerochem. 30: 15–21

Minneman, K. P., Hegstrand, L. R., Molinoff, P. B. (1979) Mol. Pharmacol. 16: 34–46

Morinan, A., Leonard, B. E. (1977) Br. J. Pharmacol. 61: 152P–153P

Murrin, L. C., Kennedy, R. H., Donnelly, T. E., Jr. (1980) Pharmacologist 22: 296

Murrin, L. C., Kuhar, M. J. (1976) Mol. Pharmacol. 12: 1082–1090

Reisine, T. D., Chesselet, M. R., Lubetzki, C., Cheramy, A., Glowisnki, J. (1982) Soc. Neurosci. Abs. 8: 526

Sherman, K. A., Zigmond, M. J., Hanin, I. (1978) Life Sci. 23: 1868–1870

Simon, J. R., Kuhar, M. J. (1975) Nature (London) 255: 162–163

Wiesel F.-A. (1976) Neurosci. Lett. 2: 35–38

Zahniser, N. R., Minneman, K. P., Molinoff, P. B. (1979) Brain Res. 178: 589–595

J. Pharm. Pharmacol. 1983, 35: 679–680
Communicated March 17, 1983

Antinociceptive effects in the rat of an adenosine analogue, N^6-phenylisopropyladenosine

MAGNUS HOLMGREN*, THOMAS HEDNAR, GUNNAR NORDBERG, TORE MELLSTRAND, *Departments of Pharmacology, Clinical Pharmacology and Anesthesiology, University of Göteborg and Sahlgren's Hospital, Göteborg, Sweden*

Purine derivatives such as adenosine occur in the central nervous system (c.n.s.) with a uniform distribution. In the c.n.s. adenosine inhibits neuronal firing, transmitter release and alters receptor sensitivity (see e.g. Stone 1981). It has been suggested that the purine derivatives as a group could form a more general synaptic control system in the c.n.s. and modulate neuronal activity. Thus, compounds in this group do not appear to act as neurotransmitters in the conventional sense (see Stone 1981).

Recently several potent adenosine analogues have been described which possess a high selectivity for membrane adenosine receptors (Fredholm 1980; Dunwiddie & Worth 1982). N^6-Phenylisopropyladenosine (PIA) is an analogue which has a relative selectivity for the A1 receptor, according to the subclassification in A1 and A2 receptors by Van Calker et al (1979) (Schwabe & Trost 1980). At the A1 site, xanthine derivatives like theophylline act as competitive inhibitors (Williams & Risley 1980; Dunwiddie & Worth 1982).

In order to investigate the potential antinociceptive effects of adenosine analogues we studied the effects of PIA on the tail flick response in rats. To further characterize the specificity of the analgesia induced via adenosine mechanisms, we also tested the reversibility by theophylline.

Methods

Adult Sprague-Dawley rats, ca 325 g, were used. Before the experiments the rats were kept in the laboratory given free access to rodent standard pellets and tap water. Each animal was used for one experiment only.

The analgesic response to PIA was determined using a tail-flick procedure (D'Amour & Smith 1941). Control response (mean of three consecutive tests) was adjusted between 2·00–3·00 s. To prevent tissue damage, cut off time in absence of a response was 7·0 s. PIA (L-form, Boehringer-Mannheim AG, Germany) was administered subcutaneously (s.c.) 0·3 mg kg^{-1}. Tail flick response was then tested each 2½ min during

* Correspondence: Department of Pharmacology, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.