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Decrease in Penbutolol Central Response as a Cause of Changes in its Serum Protein Binding

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Abstract—Penbutolol is a β-adrenoceptor antagonist that is extensively bound to α_1-acid glycoprotein (α_1-AGP), a protein that increases in inflammatory diseases thereby binding more drug in such conditions. Changes in serum binding can lead to modifications in the pharmacokinetics and pharmacodynamics of a drug, therefore, the central effect (as the anticonvulsant response) and brain uptake of penbutolol given intravenously to mice with experimental inflammation have been measured. A significant decrease of the central effect of penbutolol and its brain uptake was seen in diseased when compared with control animals ($P < 0.01$). A parallel decrease in free fraction of penbutolol in diseased vs normal animals was detected. These results suggest that there is an increase in serum binding of basic drugs related to increments in α_1-AGP concentration, which reduces their central pharmacological effect.

Drug binding to plasma protein is a limiting factor in the access of drugs to the central nervous system (CNS) (Lund et al 1972; Greenblatt et al 1980), and it is known that the drug effects are mainly a function of the free fraction rather than of the total concentration in plasma. Information is already available on the increased effect of central agents in several disease states, clearly identified as a cause of an increase of free fraction of drugs in plasma (Greenblatt & Koch-Weser 1974; Greenblatt & Allen 1978; Halliday et al 1985). However, there is an apparent lack of experimental studies regarding the effect of increased serum binding on the pharmacological effect of CNS-acting drugs. It is known that plasma α_1-acid glycoprotein (α_1-AGP), an acute phase reactant (Piafsky 1980), increases in inflammatory conditions, such as metastatic cancer or rheumatoid arthritis, and that most lipophilic amine drugs (e.g. tricyclic antidepressants, chlorpromazine, methadone) are avidly bound by α_1-AGP (Piafsky et al 1978; Abranson 1982; Teirlynk et al 1982). These drugs act on the CNS, and an increased plasma binding could decrease their brain uptake; consequently, the drug response could be affected. Thus we have set out to evaluate whether in mice with experimental inflammation, the increase in α_1-AGP plasma concentration produces a parallel increase in binding of the basic β-adrenoceptor blocking drug penbutolol, thereby decreasing its access into the brain and consequently its central effects.

Penbutolol (Heel et al 1981) was chosen because we had previously shown that it binds with high affinity to α_1-AGP, and moreover it does not bind to albumin (Aguirre et al 1988). The drug's central effect was evaluated as its anticonvulsant potency (Krall et al 1978).

Materials and Methods

Drugs

[^{3}H]Penbutolol (specific activity, 155 mCi/mg^{-1}) was a gift from Hoechst, Germany. Its radiochemical purity was over 97%. It was used as a methanol–water solution (40/60 v/v). A mixture of adequate amounts of radioactive penbutolol and pure product (also obtained from Hoechst) was used to prepare a stock solution of 25 mg mL^{-1} in saline for injection.

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Methods

In the first study, 60 male mice (28–32 g) were used to determine the anticonvulsant potency of penbutolol. Inflammation was induced in 30 mice, 48 h before the experiment, by subcutaneous injection of 0·25 mL of turpentine oil (Jamieson et al 1972). Thirty mice were injected with 0·25 mL of saline and were used as a control. All animals (5 mice per dose) received an intravenous injection, into the tail vein, of penbutolol (2·5, 3·0, 5·0, 7·0, 10 or 14 mg kg^{-1}).

Mice were maintained at a constant temperature (24–26°C) and controlled humidity (approximately 50%) with a 12 h light cycle, and had free access to food and water. All mice were subjected to the maximal electroshock seizure (MES) test, a commonly used animal model of epilepsy (Swinyard 1949), immediately after administration of penbutolol. Tonic extension of the hind limbs was used as an endpoint, and the doses necessary to block MES seizures were recorded.

In the second study, 18 male mice (28–32 g) were used to determine the brain uptake index of penbutolol. These animals were also divided in two groups, control (n = 7) and turpentine oil treated (n = 11).

All mice received [^{3}H]penbutolol as an intravenous injection of 8 mg kg^{-1} with an administration time of 30 s. Animals were decapitated 45 s after the penbutolol administration and exsanguinated; 0·3 mL of blood was obtained from each. Blood samples (0·2 mL) were collected in heparinized tubes, immediately centrifuged (20°C; 2000 *g*), and plasma (50 μL) was separated to measure total penbutolol concentration.

Brains were rapidly removed and cortex dissected according to Glowinski & Iversen (1966). Samples were accurately weighed and stored at 4°C until [^{3}H]penbutolol determination.

Samples of mouse brain (ca 100 mg) and plasma were placed in scintillation counting vials, treated with 2 mL of

Protosol solubilizer and heated at a temperature not exceeding 50°C. When they were solubilized, 10 mL of Econofluor counting solution was added to each vial and radioactivity was measured by scintillation counting using a Packard model 300-Tri-Carb Spectrometer.

Binding of [^{3}H]penbutolol (10 μg mL^{-1}) was determined in pooled (0·5 mL) plasma from control (n = 6) and turpentine-pretreated mice (n = 6) by ultrafiltration at 37°C using an Amicon MPS-1 micropartition system (March & Blanke 1985).

[^{3}H]Penbutolol free concentration was measured by scintillation spectrometry as described above.

Plasma samples from these animals were also used to evaluate α_1-AGP concentration, measured as mucoproteins (Thaw & Albutt 1980).

Statistical analysis

All data are presented as the mean ± s.e.m. Student's *t*-test was used. The criterion for significance was $P < 0.05$. The differences between the slopes of the plots which express the dose-response relationships were estimated by the method of Litchfield & Wilcoxon (1949).

Results

Figure 1 shows the effect of intravenous administration of different doses of penbutolol (2·5 to 14 mg kg^{-1}) on the electroshock-induced seizures in mice.

As we can see, the dose-response line for penbutolol is significantly shifted to the right in turpentine oil-pretreated mice, when compared with the control group, suggesting a decrease in its anticonvulsant potency. Thus, the administration of 5 mg kg^{-1} of penbutolol in control animals causes a total protection of seizures (100% of the five mice sample), but in mice with inflammation, similar doses of penbutolol

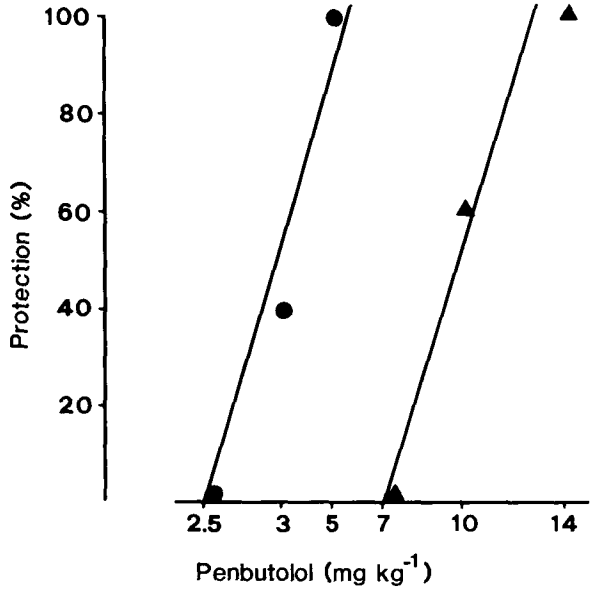

FIG. 1. Dose-response line for penbutolol. Protection against seizures is measured as the percentage of the five mice sample that did not show convulsions in presence of the corresponding dose of penbutolol. ● Control, ▲ mice with inflammation.

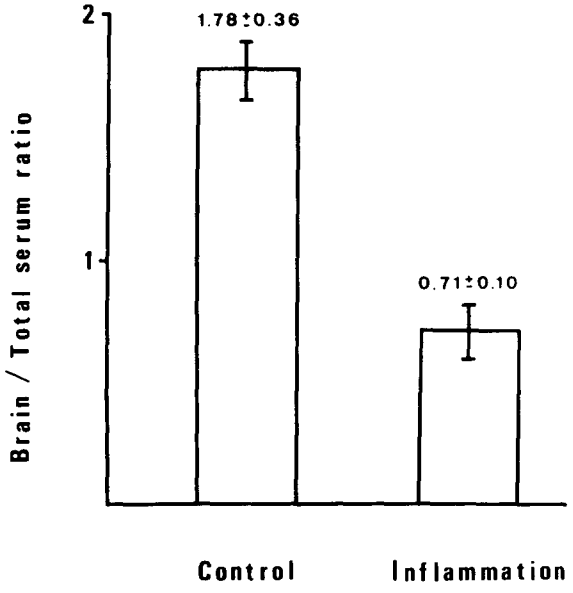

FIG. 2. Comparison of brain/total serum concentration ratio of penbutolol (μg g^{-1} tissue) after intravenous administration of 8 mg kg^{-1} in control (n = 7) and in mice with inflammation (n = 11). Results shown are means ± s.e.m.; $P < 0.01$.

Table 1. Percentage of unbound penbutolol and mucoprotein concentration in plasma from control mice and mice with experimental inflammation (mean ± s.e.m.).

Mice	Free fraction of penbutolol	Mucoprotein values (mg mL^{-1})
Control (n = 6)	8·07 ± 0·07	2·75 ± 0·07
Turpentine-oil pretreated (n = 6)	4·35 ± 0·37*	3·37 ± 0·08*

* $P < 0.001$

(range 2·5 to 7 mg kg^{-1}) show no protection. Doses of drug necessary to block electrically-induced seizures were considerably larger than in the control group (i.e. 14 mg kg^{-1} vs 5 mg kg^{-1} to produce 100% protection). Fig. 2 shows the brain uptake of penbutolol, expressed as brain/total plasma concentration ratio. As we can see, the ratio was significantly less in turpentine-oil treated animals (0·71 ± 0·10, range 0·21–1·22), when compared with the control group (1·78 ± 0·36, range 0·68–3·07; $P < 0.01$). Percentages of unbound penbutolol in plasma from normal animals and mice with experimental inflammation are shown in Table 1. The percentage of free fraction of penbutolol in control samples was 8·07 ± 0·07% vs 4·35 ± 0·37% ($P < 0.001$) in turpentine-oil pretreated mice indicating an increase in binding in the last group. Mucoprotein values are also shown in this table.

Discussion

The importance of a decrease in plasma protein binding on the effects of highly bound drugs acting on the CNS, has been verified in several studies. Most of these drugs are acidic compounds, which are mainly bound to albumin e.g.

diazepam and phenytoin; the changes observed in their central effects are mainly related to a decrease in plasma albumin concentration, observed in some pathological states (Greenblatt & Koch-Weser 1974; Greenblatt et al 1980). However, there are few reports on the opposite effect, increased drug binding, on the pharmacodynamic and pharmacokinetic behaviour of drugs.

Basic drugs are mainly bound by α_1-AGP, and the presence of several diseases can increase the concentration of this acute-phase protein and therefore, the serum binding of such drugs (Piafsky et al 1978; Piafsky 1980; Paxton 1983). Pardridge et al (1983), studied the effect of an increase in human serum binding on drug transport into the brain of rats using a carotid injection technique. These authors obtained serum from normal human subjects, and also from patients with rheumatoid arthritis and with metastatic cancer. They observed that injecting propranolol (a basic agent which binds strongly to α_1-AGP), in rat carotid, mixed with serum from patients, the brain uptake was less than when serum from normal volunteers was injected in the same manner.

Similar findings have been reported for the brain extraction of benzodiazepines, neutral drugs which are mainly bound to albumin (Jones & Wilkinson 1986). The effects of plasma protein binding on the brain uptake of these drugs was investigated in rats, also using the intracarotid artery injection technique. A variable degree of protein binding was achieved; increased binding decreased brain extraction for all the basic drugs studied. However, in both experiments (with propranolol and benzodiazepines) the authors observed that the brain extraction was less than that predicted from changes in free fraction observed in-vitro. The authors explained these experimental findings by a hypothetical mechanism of in-vivo enhanced dissociation, when compared with in-vitro data, that appears to depend on the degree of affinity of a particular drug to proteins implicated in the binding. However, in our investigation a good relation between in-vitro binding data and in-vivo brain diffusion is apparent. This situation could be explained because penbutolol is a basic agent that does not bind to albumin, in contrast with propranolol and other basic drugs. Moreover, penbutolol shows a high affinity constant to α_1-AGP, giving $nKa = 1{\cdot}6 \times 10^6$ mol^{-1} in serum from normal volunteers (Aguirre et al 1988). Binding of penbutolol increases in some pathological conditions and correlates with α_1-AGP levels (Aguirre et al 1987). These characteristics of penbutolol could explain the high in-vivo effect observed by us. In fact, our results show that after intravenous administration of penbutolol, its central effect is clearly decreased in mice with the inflammatory condition, and this change (almost two-fold) could have been predicted from in-vitro studies (Table 1). Accordingly, it is apparent that for other basic agents which are mainly and highly bound to α_1-AGP, one should consider free drug concentration in the design of dosage regimens for use in diseases where the binding of such drugs is altered.

Penbutolol is a drug with a high therapeutic index, and it only shows CNS effects when high doses are used (Turner 1983). However, it is a useful model and helps us predict, a change in dosage regimens in order to obtain the same therapeutic effect, when other CNS acting drugs (i.e.. opiate) with narrow therapeutic indices are used in diseases involving high α_1-AGP levels.

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