

Propranolol Binding in Plasma during Cardiopulmonary Bypass

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The effect of cardiopulmonary bypass on the plasma binding of propranolol was examined in seven patients. The fraction of propranolol free in plasma doubled, increasing from 6.6 to 13.5 per cent ($P < 0.001$) following the administration of heparin, 400 IU/kg. Once cardiopulmonary bypass was concluded and protamine, 8 mg/kg, given, the free fraction decreased from 13.4 to 8.7 per cent ($P < 0.005$). There was a further significant decrease to 6.5 per cent over the next 3.1 hours ($SE \pm 0.3$). Those alterations in the free fraction, which would result in more drug being available for binding to receptor sites and for exerting its pharmacologic effect, were due principally to the changes in free fatty acid levels produced by heparin and protamine, but also to the hemodilution produced by the pump prime. (Key words: Blood: anticoagulants; heparin; coagulation; protamine; plasma binding; free fatty acids. Surgery: cardiovascular; cardiopulmonary bypass. Sympathetic nervous system: sympathetic agents, propranolol.)

ALTHOUGH CONSIDERABLE ATTENTION has been given to the use of propranolol during cardiopulmonary bypass operations,¹⁻⁴ little information about the effects of cardiopulmonary bypass on the disposition and protein binding of propranolol is available. Previous studies have shown that increases in free fatty acid levels in plasma during cardiopulmonary bypass⁵ increase the fraction of phenytoin that is unbound in plasma (the free fraction). The administration of heparin prior to cardiopulmonary bypass would be expected to increase the free fatty acid levels due to an increase in lipoprotein lipase activity.⁶ The effects of a sudden increase in the levels of free fatty acids on the binding of propranolol have not previously been determined. In addition, the hemodilution that occurs when cardiopulmonary bypass is commenced may affect drug binding in plasma because of the nonphysiologic protein concentration in the pump prime.

The unbound or free fraction of the total drug in plasma is the fraction that is available for binding

to the drug's receptor sites and thus exerting its pharmacologic effect. Acute increases in the free fraction of drug in plasma will result in greater amounts of drug available for distribution outside the plasma space, with a resultant decrease in the plasma concentration and a higher volume of distribution. The importance of acute alterations in free fraction are, therefore, clear, and the aim of this study was to determine the effects of cardiopulmonary bypass on the fraction of propranolol free in plasma.

Materials and Methods

Seven patients undergoing cardiopulmonary bypass were studied after obtaining informed consent and the approval of the institutional review committee. Six of the seven patients were scheduled for elective aortocoronary saphenous bypass procedures, while one patient underwent an elective aortic valve replacement. Four of the seven patients received propranolol in the preoperative period, in doses ranging from 80 to 240 mg/day. The dose of propranolol was tapered over the 48 hours preceding operation, the last dose, 10 mg, being administered at least nine hours prior to the induction of general anesthesia. One patient undergoing aortocoronary saphenous bypass was diabetic and was receiving lente insulin, digoxin, and isosorbide dinitrate. All patients who underwent myocardial revascularization were receiving nitroglycerine, and two of these patients were also taking hydrochlorothiazide and isosorbide dinitrate. The patient who underwent aortic valve replacement needed digoxin preoperatively and received tobramycin, oxacillin and ampicillin before operation. Premedication consisted of morphine, 7.5-10 mg, scopolamine, 0.4 mg, and secobarbital, 50-100 mg, intramuscularly, 90 min before induction of anesthesia. General anesthesia was induced with diazepam, 5-10 mg, fentanyl, 0.05-0.1 mg, and ketamine, 12.5-25 mg; orotracheal intubation was facilitated with succinylcholine, 1 mg/kg. Maintenance of anesthesia was obtained using pancuronium, 0.1 mg/kg, intravenously, nitrous oxide-oxygen, 60:40 per cent and fentanyl 0.05-0.3 mg. Halothane, 0.5-2 per cent, was needed for five of the seven patients to decrease post-intubation increases in systemic arterial pressure. Ventilation was controlled using tidal volumes of approximately 10-15 ml/kg and respiratory rates of

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8–10/min to achieve optimal arterial blood-gas values. The pump was primed with Normosol-R®,§ 2,000 ml, blood, 450 ml, and Plasmanate®,¶ 250 ml. Prior to commencing cardiopulmonary bypass, heparin, 400 units/kg, was administered. After the termination of cardiopulmonary bypass and when the condition of the patient was stable, the effects of the heparin were reversed by the administration of protamine, 8 mg/kg.

Blood samples for the measurement of hematocrit, free fatty acid levels, blood-to-plasma drug concentration ratios, and the free fraction of propranolol in plasma were taken into glass tubes. The first blood sample was drawn before the administration of heparin, just before cardiopulmonary bypass was begun. A second sample was taken once cardiopulmonary bypass was established. Additional samples were obtained after the conclusion of cardiopulmonary bypass both before and after the injection of protamine. A final sample was taken when the patient returned to the surgical intensive care unit, 3.1 hours (SE \pm 0.3) after protamine administration.

Free fatty acid levels in plasma were measured by the method of Dole.⁷ Plasma binding of propranolol was measured by equilibrium dialysis of plasma, 3 ml, against phosphate buffer, 6 ml, to which has been added ³H-propranolol, 2.7 ng, in saline solution, 50 μ l, as previously described.⁸ The free fraction of drug in plasma was then calculated as the concentration of radioactive propranolol in the dialysate divided by the concentration in the plasma. The blood-to-plasma concentration ratio was measured by adding ³H-propranolol, 2.7 ng, to 1 ml blood, centrifuging, and measuring plasma radioactivity in comparison with that in 1 ml of plasma to which had been added the same amount of radioactivity.

In order to simulate the effects of hemodilution due to the pump prime, an *in-vitro* experiment was carried out in which an average total blood volume of 5 l in our patients was assumed and the effect on binding of diluting this volume of blood with a pump prime of Normosol, 2,000 ml, blood, 450 ml, and Plasmanate, 250 ml, was determined. All volumes were decreased *in vitro* a hundredfold. Thus, blood, 50 ml, was diluted by adding Normosol, 20 ml, blood 4.5 ml, and Plasmanate, 2.5 ml. The free fraction of propranolol in the plasma and hematocrit were measured before and after this dilution by the methods described previously. In addition, the binding of propranolol was measured in the two constituents of the pump prime other than blood (Plasmanate and

Normosol) by the method described, except that either Plasmanate or Normosol was substituted for plasma inside the dialysis membrane.

The effects of heparin and protamine on the binding of propranolol *in vitro* were measured by comparing the free fraction of propranolol, determined by equilibrium dialysis as described previously, in plasma containing heparin, 10 units/ml, with that in serum alone, serum plus heparin, 10 units/ml, and serum plus heparin, 10 units/ml, plus protamine, 0.33 mg/ml.

Results were analyzed using a Student *t* test for paired data, *P* < 0.05 being taken as the minimal level of significance.

Results

Following the administration of heparin, the free fraction of propranolol in plasma doubled, increasing from 6.6 to 13.5 per cent (fig. 1). There was no significant difference between the fraction of propranolol free in plasma in the samples taken after the administration of heparin and during cardiopulmonary bypass and that taken after cardiopulmonary bypass, but before the administration of protamine. Following the administration of protamine the fraction of propranolol free in plasma decreased significantly from 13.4 to 8.7 per cent. There was a further significant decrease to 6.5 per cent by the time the patients had returned to the surgical intensive care unit 3.1 hours (SE \pm 0.3) after the administration of protamine.

The administration of heparin and the establishment of cardiopulmonary bypass caused the free fatty acid levels to increase significantly from 649 to 1967 μ mol/l (fig. 2). Following the administration of protamine, the free fatty acid levels decreased significantly from 2,089 to 716 μ mol/l showing that protamine could reverse the increase in free fatty acid levels produced by heparin.

The hematocrit decreased significantly due to hemodilution by the pump prime from 39 per cent before cardiopulmonary bypass to 25 per cent during bypass (fig. 3). During the remainder of the study the hematocrit gradually increased towards normal.

There was a significant correlation between the fraction of propranolol free in plasma and the ratio of propranolol in the blood to that in the plasma (fig. 4). Because more propranolol was free in Plasmanate (31 per cent \pm 0.2), and all was free in Normosol (100 per cent \pm 1), simulation of hemodilution by the pump prime *in vitro* produced a decrease in hematocrit from 45 to 34 per cent while the free

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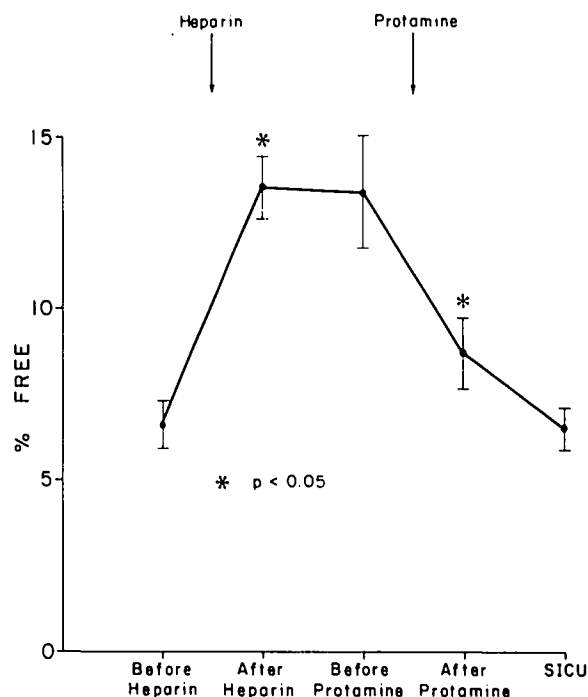


FIG. 1. Free fractions (per cent) of propranolol (\pm SEM), before, during and after cardiopulmonary bypass. Comparisons are with immediately preceding values.

fraction of propranolol in plasma increased from 10.3 to 14.1 per cent. The effects of heparin and protamine on propranolol binding measured *in vitro* showed that the free fraction of propranolol in serum alone ($9.2 \text{ per cent} \pm 0.8$) was not altered by the addition of heparin ($10.4 \text{ per cent} \pm 1.2$) or by the addition of heparin and protamine ($10.6 \text{ per cent} \pm 1.2$), nor was it different from that in heparinized plasma ($9.2 \text{ per cent} \pm 1.7$).

Discussion

Protein binding of propranolol measured by the method used here reflects its degree of binding throughout the therapeutic range of plasma propranolol concentrations. Because it is not feasible to measure drug binding *in vivo*, an *in-vitro* method was used. This method allows the best available approximation to the *in-vivo* situation, the assumption being that the plasma binding of drug *in vivo* is the same as the measured *in-vitro* binding. This study showed that in our patients the fraction of propranolol free in plasma doubled during cardiopulmonary bypass. The fact that changes in binding correlated with changes in free fatty acids suggests that the change in propranolol binding was due to the increased levels of free fatty acids following the administration of heparin displacing propranolol from its protein-binding sites. An additional factor that contributed to

the change was the hemodilution produced by the pump prime, the constituents of which, other than blood, bound propranolol less than plasma. It was possible to simulate *in vitro* the effects of hemodilution by the pump prime. With a decrease in hematocrit very close to that which occurred in our patients, there was an increase in the free fraction of propranolol of only a third, whereas during cardiopulmonary bypass in our patients the free fraction doubled, because of the decrease in hematocrit and an increase in levels of free fatty acids. An additional finding that leads us to believe that the increase in free fatty acids was principally responsible for the change in free fraction was the decrease in free fraction that occurred following the administration of protamine, with only a slight increase (2.3 per cent) in hematocrit. It is thought that heparin increases free fatty acid levels by releasing lipoprotein lipase⁶; therefore, it is not surprising that heparin and protamine *in vitro* had no effect on propranolol binding.

Although changes in free fatty acid levels have previously been shown to affect the free fraction of

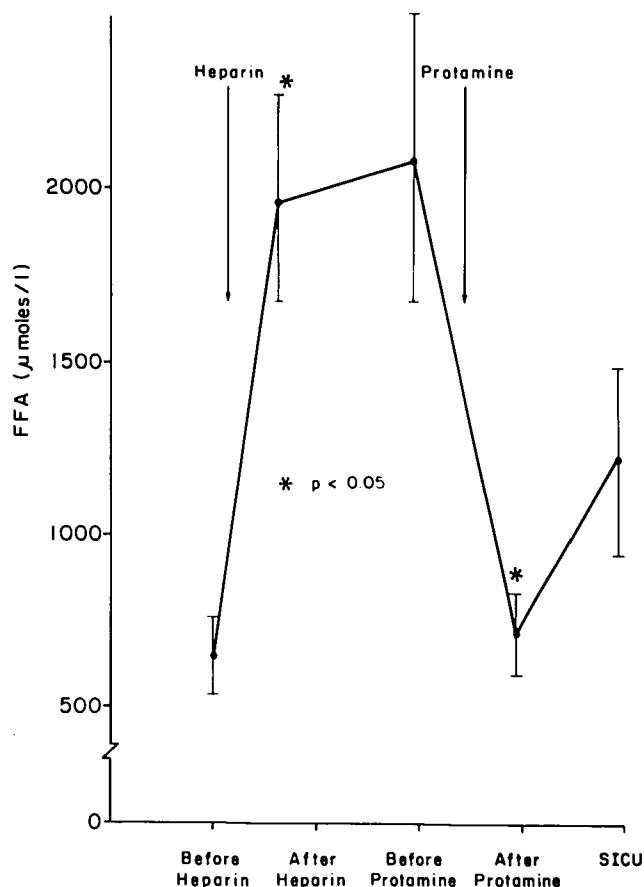


FIG. 2. Free fatty acid levels ($\mu\text{mol/l}$), (\pm SEM), before, during and after cardiopulmonary bypass. Comparisons are with immediately preceding values.

phenytoin,⁵ the change produced in the free fraction of propranolol suggests that this phenomenon involves a much wider spectrum of drugs than had previously been recognized. The implications of a doubling of the free fraction of drug are important. Firstly, this will make more drug available for binding to receptor sites and so increase the pharmacologic effect of propranolol. In the case of propranolol, this might result in patients requiring unexpectedly high doses of isoproterenol or other betareceptor stimulants at the termination of cardiopulmonary bypass. In addition, more of the drug will be available for distribution to sites outside the plasma, causing an increased volume of distribution. Thus, more drug can enter the erythrocytes, accounting for the increase in blood-to-plasma ratio with increasing free fraction found in this study. Acute redistributional changes such as this will result in an initial lowering of plasma propranolol concentrations and may account for the rapid decrease in propranolol levels seen when cardiopulmonary bypass is begun, which previous authors** have incorrectly related to hemodilution by the pump prime. The effect of hemodilution by the pump prime on total propranolol levels can be predicted as:

$$\Delta C_{ss} = C_{ss} \times \frac{V_{pp}}{V_1 + V_{pp}}$$

Where ΔC_{ss} is the change in drug concentration; V_{pp} is the volume of the pump prime (2.7 l); C_{ss} is the drug

** Hug CC Jr, Kaplan JA, Rigel EP: The effect of cardiopulmonary bypass on plasma propranolol concentrations and response to isoproterenol (abstr). American Society of Anesthesiologists, Annual Meeting, October 1977, p 501.

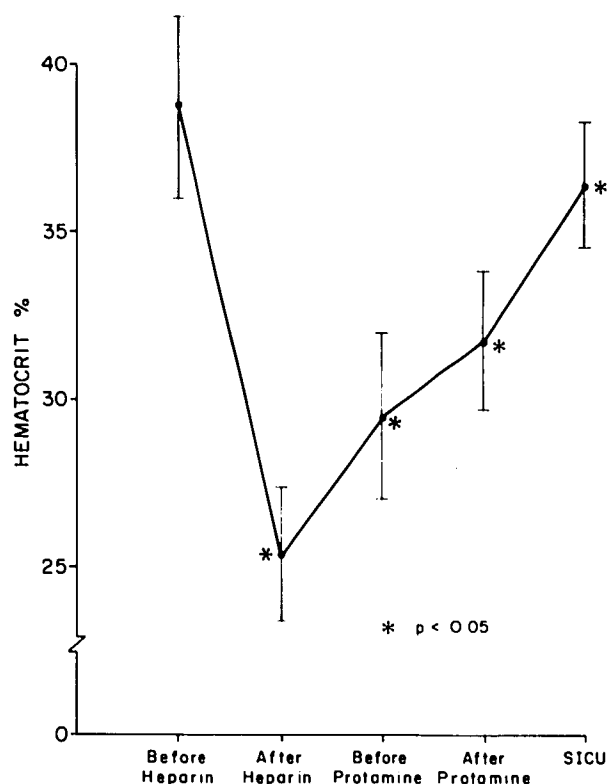
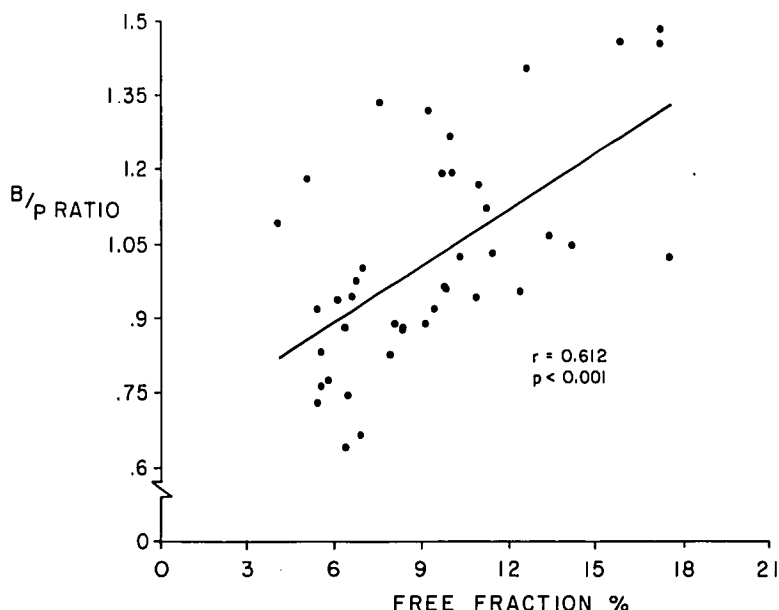


FIG. 3. Hematocrits (per cent), (\pm SEM) before, during, and after cardiopulmonary bypass. Comparisons are with immediately preceding values.

concentration prior to hemodilution; V_1 is the volume of distribution of the α phase (approximately 70 l). Thus, hemodilution by the pump prime would produce a decrease in total propranolol levels of less than 4 per cent.

FIG. 4. Blood/plasma (B/P) ratios and free fractions (per cent) during cardiopulmonary bypass.



The effects that altered plasma binding have on drug elimination depend very much on the drug in question. In the case of propranolol, elimination is unaffected by plasma binding (unrestrictive elimination), so that following the redistribution that occurs with a decrease in protein binding, total plasma levels will return to their initial levels, with continued oral administration, resulting in a permanent increase in free drug concentration.⁹ In patients undergoing cardiopulmonary bypass the long-term effects on elimination will not be so important, as the binding changes are reversed by protamine, but they may be significant in patients receiving long-term heparin therapy.

This study has re-emphasized the importance of measuring the fraction of drug free in plasma, and while we have examined only the effects of cardiopulmonary bypass on propranolol binding in plasma, the binding to protein of other drugs that are administered during this procedure should be evaluated. In addition, because of our subsequent finding that even a low dose of heparin¹⁰ can alter drug binding, other clinical situations in which heparin is administered should be examined.

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