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Benzodiazepine Receptor–Mediated Chemotaxis of Human Monocytes

Abstract. *Benzodiazepines, which are widely prescribed for their antianxiety effects, are shown to be potent stimulators of human monocyte chemotaxis. The chemotactic effects of benzodiazepine receptor agonists were blocked by the peripheral benzodiazepine receptor antagonist PK-11195, suggesting that these effects are mediated by the peripheral-type benzodiazepine receptor. Diazepam was also active in inducing chemotaxis. Binding studies on purified monocytes revealed high-affinity peripheral benzodiazepine receptors, and the displacement potencies of various benzodiazepines correlated with their relative potencies in mediating chemotaxis. The demonstration of functional benzodiazepine receptors on human monocytes, together with recent evidence of receptor-mediated monocyte chemotaxis by other psychoactive peptides (such as opiate peptides), suggests a biochemical substrate for psychosomatic communication.*

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The benzodiazepines are among the most widely used of all drugs (*1*) and are commonly prescribed for their anxiolytic, hypnotic, and anticonvulsant properties (*2*). These behavioral and neurological effects of benzodiazepines are mediated through high-affinity, stereoselective receptors that are almost exclusively localized to the central nervous system (CNS) (*2, 3*). In addition to identifying CNS benzodiazepine receptors, radioreceptor assays have revealed another class of benzodiazepine recognition sites broadly distributed in many non-neuronal tissues, including kidney, heart, platelets, mast cells, adrenals, as well as several cultured cell lines (*3, 4*). These binding sites have provisionally been defined as "peripheral" benzodiazepine receptors since structure-activity studies have demonstrated marked differences between them and CNS or "central" benzodiazepine receptors. For example, Ro5-4864 (4-chlorodiazepam) is one of the most potent ligands for the peripheral benzodiazepine receptor, yet it is virtually inactive at the central benzodiazepine receptor. In contrast, clonazepam, one of the most potent behaviorally active benzodiazepines, binds with high affinity to the central benzodiazepine receptor but is essentially inactive at the peripheral receptor. Diazepam (Valium), the most commonly prescribed benzodiazepine, binds with relatively high affinity to both the peripheral and central receptors.

Despite the many reports characterizing benzodiazepine receptors on various tissues, the physiological function or functions of the peripheral receptor are largely unknown, although several effects have been observed in vitro. Benzodiazepines have been reported to inhibit mitogenesis in Swiss mouse 3T3 fibroblasts (*5*), to promote differentiation of Friend erythroleukemia cells (*5, 6*), to induce melanogenesis in B16/C3 melanoma cells (*7*), to inhibit the growth of thymoma cells in vitro (*8*), and to decrease cardiac muscle contractility (*9*). These effects, in some cases (*7–9*), have correlated with the presence of peripheral benzodiazepine receptors on these tissues.

We examined the effects of benzodiazepines on the chemotaxis of human monocytes. Monocytes or their noncirculating counterparts, macrophages, are a heterogeneous population of cells that subserve key roles in immune system function and figure prominently in many aspects of tissue repair and restructuring, inflammation, and antineoplastic defense (*10*). Agents that are chemotactic for monocytes include bacterial products, peptide fragments derived from complement or clotting activation, factors from other immune system cells, as well as proteolytic fragments of elastin, fibronectin, and collagen, leukotrienes or other prostaglandin metabolites, and numerous small peptides of diverse origin (*11*). Recently, neuropeptides such as

Table 1. Checkerboard analysis for chemotactic response of monocytes to Ro5-4864, a benzodiazepine attractant. The data represent the number of migrating cells per field ± standard error of the mean (n = 3) as described (*13*). Various concentrations of Ro5-4864 as indicated were placed in both upper and lower chemotaxis chambers.

Concentration in lower chamber (M)	Concentration in upper chamber (M)			
	0	10^{-13}	10^{-12}	10^{-11}
0	40 ± 8	32 ± 5	39 ± 7	31 ± 5
10^{-13}	90 ± 6	56 ± 6	60 ± 8	66 ± 4
10^{-12}	126 ± 5	77 ± 6	66 ± 11	61 ± 4
10^{-11}	175 ± 5	85 ± 8	71 ± 7	53 ± 5

β-endorphin, substance P, and bombesin, which are not currently considered to be inflammatory reaction products, were reported to be chemotactic for monocytes (*12*). These results suggested that other behavior- and mood-modifying drugs may also have influences on immune function. We now report that benzodiazepines are potent promoters of human monocyte chemotaxis and that this effect is mediated through a peripheral benzodiazepine receptor.

Chemotaxis was evaluated with the use of blind-well chemotaxis chambers (Neuroprobe), in which cells are allowed to migrate through membrane filters in response to various attractants (*13, 14*). Migrating cells were counted microscopically in three fields in triplicate with an optical image analyzer (*14*). Figure 1 shows the effect of the prototypical peripheral benzodiazepine receptor ligand Ro5-4864 in promoting migration of partially purified human monocytes. The apparent median effective dose (ED_{50}) for Ro5-4864–induced chemotaxis was approximately $10^{-13}M$. Higher concentrations ($>10^{-8}M$) resulted in a diminished chemotactic response. In contrast, the central benzodiazepine receptor agonist, clonazepam, was without effect when tested under identical assay conditions over a broad range of concentrations (Fig. 1). Diazepam, which binds to both subtypes of benzodiazepine receptor, was also active in promoting monocyte chemotaxis (Fig. 1), with an ED_{50} of $2 \times 10^{-12}M$. These results suggest that the benzodiazepine receptor that mediates monocyte chemotaxis is of the peripheral type. The enhanced migration we observed could be due to an increase in random migration (chemokinesis) or migration in response to a gradient (chemotaxis). The chemotactic nature of the response to Ro5-4864 (Table 1) was determined by a "checkerboard" analysis (*11*), which includes attractants in both upper and lower compartments of the chamber. The results showed that monocytes migrate primarily in response to

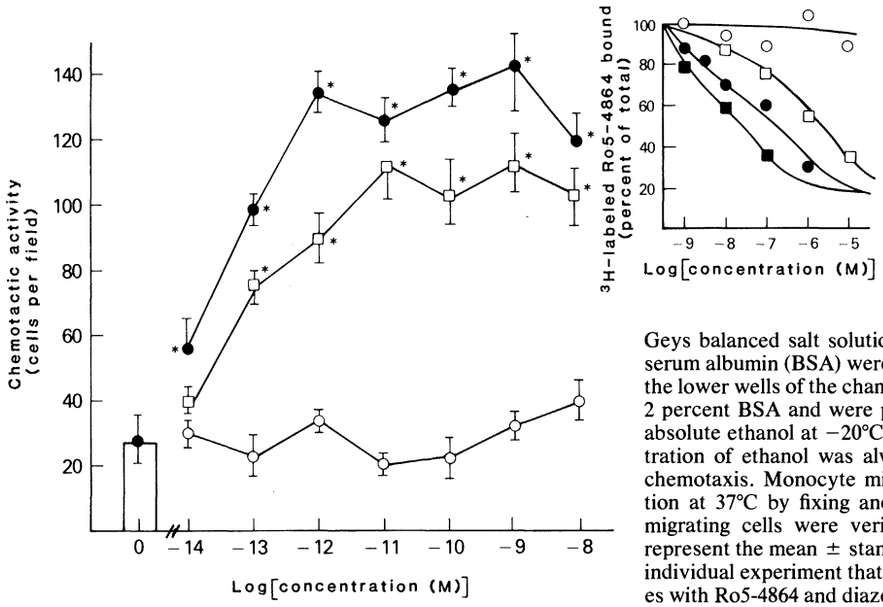
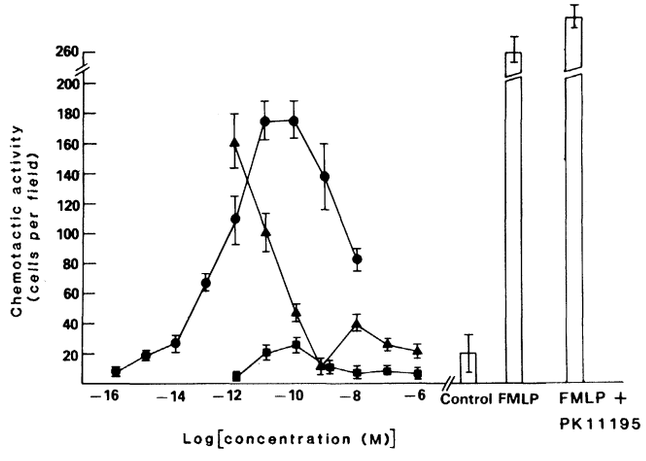


Fig. 1. The effects of various benzodiazepines on the chemotaxis of human peripheral blood mononuclear cells. Mononuclear cells, obtained from heparinized blood from healthy human volunteers, were partially purified by sedimentation over Ficoll-Paque (Pharmacia) (*15*). This population of cells was comprised of approximately 30 percent monocytes and 70 percent lymphocytes as determined by esterase and specific antibody staining. The upper and lower compartments of the chemotaxis chambers were separated by a polycarbonate membrane (Nuclepore; pore size, 5 μm) (*13*). Mononuclear cells (5.5×10^4) in Geys balanced salt solution (GBSS; *p*H 7.4) containing 2 percent bovine serum albumin (BSA) were placed in the upper wells and test attractants in the lower wells of the chambers. All drugs were diluted in GBSS containing 2 percent BSA and were prepared from a $10^{-4}M$ stock solution stored in absolute ethanol at −20°C and diluted just before assay. The final concentration of ethanol was always less than 1 percent and had no effect on chemotaxis. Monocyte migration was assessed after a 90-minute incubation at 37°C by fixing and staining the membrane-adherent cells. These migrating cells were verified as monocytes morphologically. The data represent the mean ± standard error of the mean of the cell migration of an individual experiment that was replicated with similar results. The responses with Ro5-4864 and diazepam were significantly different from control (*) ($P < 0.01$, *t* test). (Inset). Displacement of specific ^{3}H-labeled Ro5-4864 bound to purified monocytes by various drugs. Displacement potencies of Ro5-4864, PK-11195, diazepam, and clonazepam were determined by incubating 4×10^6 monocytes in 0.05*M* tris and 0.15*M* NaCl, *p*H 7.4 (final volume, 0.5 ml) with ^{3}H-labeled Ro5-4864 (4 n*M*; specific activity, 82.7 Ci/mmol) and various concentrations of unlabeled drug as described (*23*). After a 45-minute incubation at 0° to 4°C, the reactions were terminated by rapid vacuum filtration of the solution over GF/B glass fiber filters (Whatman) and then by washing the filters twice with 5.0 ml of ice-cold buffer (*24*). Specific binding, defined as the total binding minus that observed in the presence of $10^{-5}M$ unlabeled Ro5-4864 (nonspecific binding), was approximately 75 percent at a ligand concentration of 4 n*M*. Displacement curves for each drug were computed as a percentage of the maximal specific ^{3}H-labeled Ro5-4864 binding in the presence of each drug over the indicated concentration ranges. IC_{50} values were estimated from displacement curves such as those shown here. Values are from a typical experiment repeated three times with similar results. Symbols: (●) Ro5-4864, (□) diazepam, (○) clonazepam, and (■) PK11195.

Fig. 2. Reversal of Ro5-4864–induced chemotaxis by the benzodiazepine antagonist PK-11195 in purified monocytes prepared by centrifugal elutriation from human peripheral blood mononuclear cells (*15*). Lower chambers contained only Ro5-4864 or PK-11195 at various concentrations. Where antagonist effects were to be evaluated, a combination of Ro5-4864 ($10^{-10}M$) and various concentrations of PK-11195 were added before testing. The *N*-formyl peptide attractant FMLP was also tested by itself ($10^{-8}M$) or in combination with PK-11195 ($10^{-8}M$) to demonstrate antagonist specificity. Control incubations consisted of buffer only. Data are expressed as the mean chemotactic activity (which represents the actual number of migrating cells) ± standard error of the mean and are from a representative experiment that was repeated several times with similar results. The responses for Ro5-4864 at concentrations from $10^{-8}M$ to $10^{-13}M$ were significantly different from control ($P < 0.005$, *t* test). PK-11195 ($10^{-6}M$ to $10^{-12}M$) alone had no significant effect on chemotaxis but produced a significant inhibition of the Ro5-4864 ($10^{-10}M$) chemotactic response at concentrations from $10^{-6}M$ to $10^{-11}M$. Symbols: (●) Ro5-4864, (■) PK11195, and (▲) PK11195 + Ro5-4864 ($10^{-10}M$).



a positive concentration differential, which is an indicator of chemotaxis. A slight chemokinetic effect at higher attractant doses was observed as well.

That the effects of benzodiazepines on monocyte chemotaxis are mediated through peripheral benzodiazepine receptors is supported by binding studies on elutriator-purified (*15*) (>95 percent) monocytes (Fig. 1, inset). High-affinity and saturable binding sites for ^{3}H-labeled Ro5-4864 were demonstrated on purified monocytes, and the displacement potencies of various benzodiazepines indicated a peripheral benzodiazepine receptor on the human monocyte (Fig. 1). PK-11195, a peripheral ligand (*16*) with receptor antagonist activity, was the most potent of the benzodiazepines in displacing specific ^{3}H-labeled Ro5-4864 bound to monocytes, with a median inhibition concentration (IC_{50}) of $7.5 \times 10^{-9} M$; Ro5-4864 was only slightly less effective (IC_{50}, $5 \times 10^{-8} M$); diazepam was less potent (IC_{50}, $7 \times 10^{-7} M$); and clonazepam was inactive at concentrations up to 10 μM. Thus, the binding data are in agreement with the pharmacological data on benzodiazepine-induced chemotaxis, and they support the notion that specific receptors for benzodiazepines exist on human monocytes that mediate the chemotactic response of these cells.

To characterize further the monocyte benzodiazepine receptor and to provide evidence for the specificity of the chemotactic response, we examined the effects of the putative peripheral benzodiazepine receptor antagonist PK-11195 on chemotaxis with the use of purified monocytes. In these experiments, increasing concentrations of PK-11195 were added to a fixed concentration of Ro5-4864 ($10^{-10} M$) before testing. The results (Fig. 2) showed that PK-11195 alone, although devoid of any action on monocyte chemotaxis, inhibited the migration induced by Ro5-4864. Approximately $10^{-11} M$ PK-11195 blocked chemotaxis induced by Ro5-4864 ($10^{-10} M$) by 50 percent; this potency ratio corresponds with the relative affinities of these drugs in competing for specific ^{3}H-labeled Ro5-4864 bound to monocytes (Fig. 1, inset). PK-11195 also reversed diazepam-induced monocyte chemotaxis. At equimolar (10^{-8}) concentrations, PK-11195 did not diminish the response to the chemoattractant peptide formylmethionyl leucine phenylalanine (FMLP), which further demonstrates the specificity of these responses (Fig. 2).

Thus, as judged from the results of these in vitro methods, some benzodiazepines appear to be potent chemotactic agents, and this effect is shared by diazepam, the most commonly used antianxiety drug. The effects of benzodiazepines on monocyte chemotaxis appear to be mediated by peripheral benzodiazepine receptors in that Ro5-4864 (but not clonazepam) was a potent agonist, and PK-11195 was a potent antagonist, of the benzodiazepine-induced chemotactic response. While the binding affinities of Ro5-4864, PK-11195, and diazepam for intact monocytes were observed in the usual nanomolar (*5–9*) range, chemotaxis induced by these drugs is readily detectable in the picomolar range. This apparent difference may reflect the requirement of the monocyte benzodiazepine receptor for a low percentage (<1 percent) of occupancy to mediate chemotaxis or its ability to alter its affinity under various conditions (*17*).

The role of benzodiazepines in monocyte and immune system function in vivo is unknown. Several laboratories have recently provided evidence for the existence of endogenous peptide ligands for benzodiazepine receptors (*18*). Such an endogenous ligand, released locally, could serve to recruit subpopulations of monocytes to specific body sites where these cells and their products could function in inflammation and tissue repair as well as in modulation of other immunological and endocrine parameters. The effects of benzodiazepines on monocyte function may not be solely limited to chemotaxis since chemoattractants often regulate other aspects of monocyte physiology as well (*11*). Tissue regeneration, for example, is regulated in part by monocyte-released growth hormones (*19*). These data for benzodiazepine-induced monocyte chemotaxis, coupled with previous reports demonstrating that in some cases benzodiazepines promote differentiation and inhibit cell proliferation (*5–8*), raise the possibility that benzodiazepines such as Ro5-4864 and diazepam may have growth-controlling effects.

Neuropeptides have been studied for their behavioral and neuroendocrine effects (*20*) and are increasingly being shown to exert effects on immune system function (*21, 22*). Because these ligands and their receptors are richly distributed in brain regions that mediate emotion and higher cognitive functions, it seems plausible that the same neurohumoral mediators of various mood states in brain may also communicate to monocytes and other cells involved in healing and homeostatic processes (*22*). Short signal peptides (neuropeptides) and their surface receptors define a group of cells whose function may be to integrate information from the central nervous, immune, and endocrine systems through a psychoimmunoendocrine network, thereby altering the behavior of the whole organism.

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