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Leukocyte mobilization from the guinea pig spleen by muscarinic cholinergic stimulation

G. Sandberg

Lymphocyte Unit, Department of Neuroscience, Karolinska Institute, Stockholm (Sweden)
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Abstract. Important interactions between the immune system and the nervous and endocrine systems have become increasingly accepted. The present results demonstrate that the cholinergic agonist carbacholine greatly increased the number of granulocytes and lymphocytes in the splenic venous blood, but not arterial blood, shortly after administration to guinea pigs. The effect was largely blocked by pretreatment with atropine. In contrast, animals treated with indomethacin had a decreased number of leukocytes in both splenic venous and arterial blood. A decrease in relative splenic weight due to carbacholine treatment was also blocked by atropine. However, cholinergic leukocyte mobilization, or that previously observed after adrenergic stimulation, may not be caused by capsule contraction since it is not accompanied by mobilization of erythrocytes. Furthermore, indomethacin, which potentiates the response of splenic smooth muscle to adrenergic stimuli, blocked the effect of noradrenaline (NA) on leukocyte mobilization.
Key words. Spleen; guinea pigs; leukocyte mobilization; cholinergic.

The number of lymphocytes in splenic venous blood of guinea pigs normally exceeds that in splenic arterial blood, reflecting a continuous production and release of newly formed cells[1], including immune specific cells formed after immunization[2]. Both B and T lymphocytes are normally released into the blood[3,4]. Rapid mobilization of leukocytes from the spleen occurs after treatment with adrenaline, noradrenaline (NA), isoprenaline or theophylline[5,6]. The mechanisms of these effects are poorly understood, but may involve changes in cell adhesion, cell migration, and blood flow, or may be due to splenic contraction. In the present work the effects of the cholinergic agonist carbacholine (which stimulates nicotinic and muscarinic receptors like the natural ligand, acetylcholine, but is not as easily degraded by acetylcholine esterase) were studied in normal animals and in animals pretreated with atropine (a blocker of muscarinic receptors). The effect of indomethacin (a potent inhibitor of prostaglandin synthesis) was studied in normal and NA-treated animals to examine the role of prostaglandins on the well-known lymphocyte-mobilizing effect of adrenergic stimulation. The investigation was made to shed further light on the regulation of leukocyte output from the spleen – an important component of the immune system.

Materials and methods

A total of 92 male guinea pigs (pigmented strain) weighing 250–300 g were used for the studies. Before examination the animals were anaesthetized with pentobarbital sodium (25 mg/kg, intraperitoneally). Carbacholine (3.1 μg) or NA (50 μg) were injected intracardially (during anaesthesia) in a volume of 50 μl physiological saline 5 min before investigation. Animals were pretreated with indomethacin (2.5 mg) or atropine (250 μg) by intraperitoneal injections 1 h before examination. Controls received only saline or were untreated. The two control groups, which did not differ, were pooled and are presented together. At examination, the animals were weighed and the splenic region exposed by incision between the two most caudal ribs on the left side. A small incision with microscissors into one splenic vein allowed the collection of 25 μl venous blood with a heparinized pipette. Immediately afterwards, the same amount of arterial blood was collected by the same procedure. The number of mononuclear cells (mainly lymphocytes) and polynuclear cells (mainly neutrophilic granulocytes) were determined by counting in a Bürker chamber. The weight of the spleen was then determined. Differences between groups of animals were evaluated statistically with Student's t-test (if not stated otherwise), and the veno-arterial differences in cell number with the paired t-test and two non-parametric tests (two sample sign test for equal medians; Wilcoxon test for matched pairs). The statistical analysis was performed using computer software SOLO (BMDP Statistical Software, Inc).

Results

Splenic weight. Animals treated with carbacholine alone had a reduced relative splenic weight (table 1). The effect was inhibited by pretreatment with atropine. Furthermore, an increase in splenic weight after atropine pretreatment was suggested by the results (although values were not significantly different from controls).

Table 1. Effect of carbacholine and atropine on relative splenic weight.

Treatment	n	Splenic weight (mg/g body weight)
Controls	21	1.69 × 0.09
Carbacholine	22	1.50 ± 0.07
Atropine	11	1.94 ± 0.17
Atropine + carbacholine	16	2.04 ± 0.16
Indomethacin	12	1.63 × 0.08
Indomethacin + NA	10	1.64 × 0.11

Atropine (250 µg) or indomethacin (2.5 mg) was given intraperitoneally 1 h before investigation, and carbacholine (3.1 µg) or NA (50 µg) was injected intracardially 5 min before investigation. Splenic weight was determined after collection of blood for leukocyte counting (n = number of animals). Mean ± SEM.
Carbacholine caused significant ($p < 0.05$; Mann Whitney) reduction in splenic weight. The effect was significantly ($p < 0.01$) blocked by atropine. Atropine alone tended to increase splenic weight.

Splenic venous blood. Increased numbers of granulocytes and lymphocytes were observed in animals treated with carbacholine (fig. 1). Atropine had no effect in itself, but largely blocked the effect of carbacholine. In contrast, animals treated with indomethacin had reduced numbers of granulocytes and lymphocytes in splenic efferent blood. Indomethacin blocked the previously demonstrated ability of NA to increase the outflow of leukocytes. The venous hematocrit was not affected by any treatment used (not shown).
Arterial blood. Significant leukopenia was induced by indomethacin (fig. 2). The arterial hematocrit was not affected by any treatment used (not shown).
Veno-arterial differences. Control animals showed a higher veno-arterial difference in lymphocytes than in granulocytes. There was also a slight difference in hematocrit (table 2).

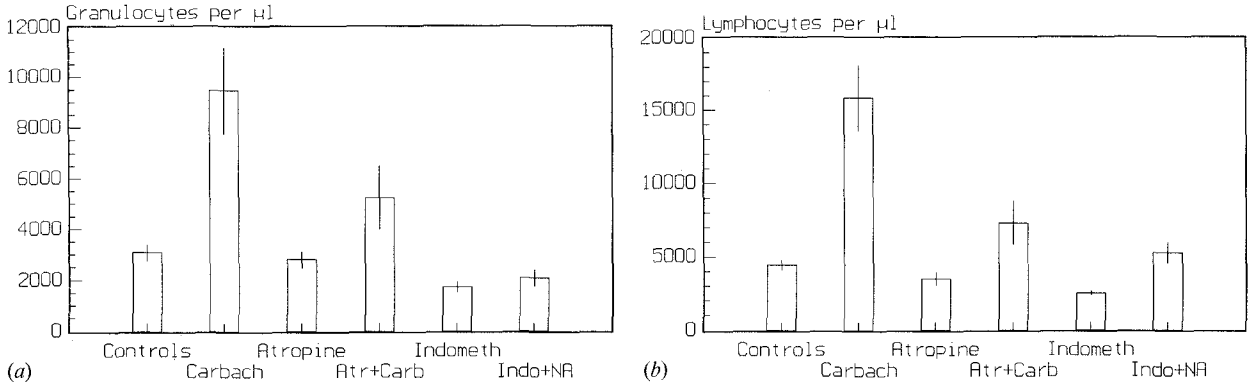

Figure 1. Number of granulocytes (*a*) and lymphocytes (*b*) per µl of splenic venous blood (±SEM). The different groups were treated as described in the legend to table 1. Atr, atropine; carbach and carb, carbacholine; indometh and indo, indomethacin. Carbacholine increased the number of both granulocytes and lymphocytes ($p < 0.001$). The effect was significantly blocked by atropine ($p < 0.05$ and $p < 0.01$, respectively). In contrast, the level of both granulocytes and lymphocytes was reduced by indomethacin ($p < 0.001$).

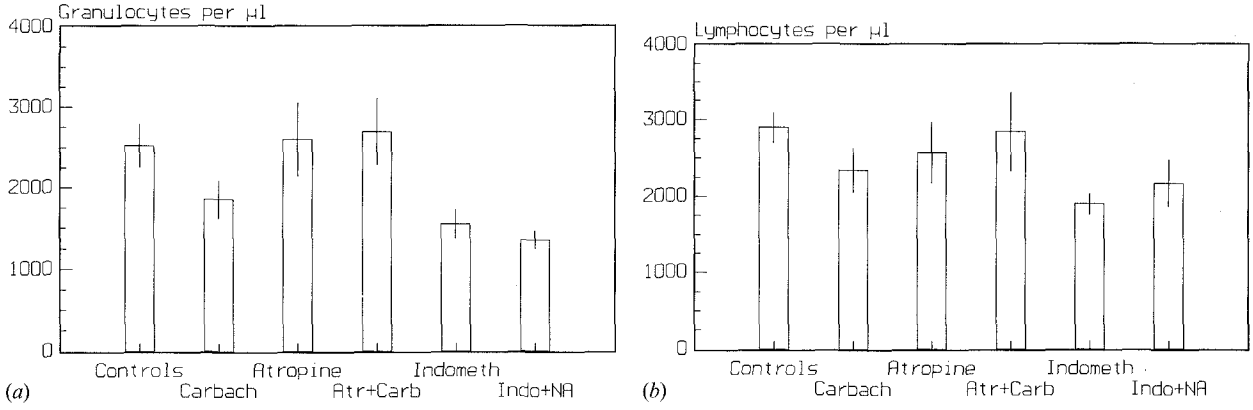

Figure 2. Number of granulocytes (*a*) and lymphocytes (*b*) per µl of splenic arterial blood (±SEM). The different groups were treated as indicated in Table 1. Atr, atropine; carbach and carb, carbacholine; indometh and indo, indomethacin. Indomethacin reduced the number of both granulocytes and lymphocytes ($p < 0.01$), even in combination with NA. No other statistically significant effects were observed.

Table 2. Splenic veno-arterial differences in number of granulocytes, lymphocytes and in hematocrit.

Group	Granulocytes	Lymphocytes	Hematocrit
Controls	557 ± 186*	1532 ± 329***	0.9 ± 0.3*
Carbacholine	7812 ± 1597***	13849 ± 2435***	−0.7 ± 0.9
Atropine	209 ± 308	918 ± 394	2.2 ± 0.8*
Atropine + carbacholine	2545 ± 941**	4471 ± 1053***	−0.1 ± 0.2
Indomethacin	162 ± 86	613 ± 91***	0.1 ± 0.3
Indomethacin + NA	710 ± 328	3104 ± 667**	0.6 ± 0.3

The different groups were treated as described in Table 1. *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$) indicate a statistically significant veno-arterial difference. The values were obtained by Student's t-test and at least one non-parametric test.
The granulocyte and lymphocyte values were significantly increased by carbacholine ($p < 0.001$) and this effect was significantly blocked by atropine ($p < 0.01$ and $p < 0.001$, respectively). The lymphocyte difference was also significantly increased after indomethacin + NA ($p < 0.02$). A significantly reduced value was obtained after treatment with indomethacin alone ($p < 0.01$).

Carbacholine significantly increased the export of both granulocytes and, particularly, of lymphocytes. There was no significant effect on the hematocrit. The effect of carbacholine was largely inhibited by atropine, and did not remain after 2 h (results not shown). One h after atropine the venous hematocrit was slightly higher than in arterial blood (table 2), but the difference was not significantly higher than that in control animals.

Discussion

Cholinergic innervation of the spleen of various species seems to be scarce or absent[7–10]. However, cholinergic drugs cause contraction of capsular muscle cells via muscarinic receptors, decreased splenic volume[11,12], and vasodilation[7,13]; higher doses may cause vasoconstriction instead due to stimulation of nicotinic receptors with undefined localization[7,10]. The differences in response of splenic vascular and non-vascular smooth muscle was emphasized by Davies and Withrington[7]. Many of the muscarinic cholinergic receptors in the spleen may be presynaptic elements of sympathetic nerves[14] and it has been suggested that they mediate inhibition of NA release[15,16]. In addition to the effects on smooth muscle cells, cholinergic stimulation may enhance both cellular and humoral immune reactions in the spleen[17,18].

In the present work, carbacholine caused a reduction in splenic weight (relative to body weight) which was antagonized by atropine. This indicates that a muscarinic mechanism influences the content of blood in the spleen, either by increasing the tonus of capsular muscle cells, or by altering the balance between afferent and efferent blood flow. The effect of carbacholine on splenic venous blood cells in the present work was also inhibited by atropine, so muscarinic receptors in the spleen are probably also involved in this case.

Since alpha- and beta-adrenoreceptors in muscle cells of the splenic capsule also mediate splenic contraction[19,20] and cause mobilization of splenic leukocytes[5], this could be the common mechanism for the actions of all these different stimuli. However, some facts indicate that another mechanism may be involved in splenic leukocyte mobilization. Adrenaline causes lymphocytosis, mainly due to increase of B-lymphocytes[21]. Careful studies showed that adrenaline-induced lymphocytosis was biphasic and followed by a later increase in granulocytes; neither the spleen nor the thoracic duct seemed to be primarily responsible for these effects[22]. Therefore, the mobilization of leukocytes from the spleen by adrenaline is not caused by a spleen-specific mechanism. The selective effect on leukocytes in the present work, with no increase in hematocrit, also argues against the contraction hypothesis. Catecholamines may also cause selective release of specific subsets of lymphocytes, which is difficult to explain by an effect on splenic smooth muscle cells[23]. Furthermore, lymphocytosis is also caused by splenectomy, indicating the presence of other important lymphocyte mobilizing sites[24–26].

Lymphocytes of humans, rats and mice contain muscarinic receptors[27], although these receptors may differ from those in the brain[28,29]. Ado and Dontsov[30] reported on acetylcholine-induced mobilization of spleen lymphocytes. Adrenergic receptors on leukocytes have previously been demonstrated (see review by Chambers et al.[31]). This indicates the possiblity of a direct action on splenic leukocytes, perhaps altering migration of adhering properties.

In the guinea pig, as in most species, the splenic nerve contains mainly adrenergic sympathetic fibres innervating the capsule, trabecules and blood vessels[32]. At least in rats and mice, adrenergic nerves are also in close contact with cellular elements of the spleen[33], particularly in the white pulp[34,35]. Nerve stimulation in general causes an early decrease in splenic volume and a later vasoconstriction[7].

Indomethacin has been reported to potentiate the response of splenic smooth muscle to adrenergic stimuli[36]. This also argues against a role of splenic smooth muscle in mobilization of splenic leukocytes, since indomethacin in our experiments blocked the effect of NA. This finding also suggests an important role for prostaglandins in splenic leukocyte mobilization, at least as far as NA is

concerned. In accordance with this, Ulich et al.[37] found evidence for a prostaglandin-induced leukocyte release from the spleen of rats. They suggested this was due to diminished adherence, and in agreement with this indomethacin increases adherence of leukocytes in postcapillary venules[38]. A possible effect of indomethacin on cell migration in vivo is also suggested by results showing that prolonged indomethacin treatment affected the number of leukocytes in the spleen[39].

1 Ernström, U., and Sandberg, G., Scand. J. Haemat. *9* (1972) 387.
2 Ernström, U., and Söder, O., Scand. J. Immun. *3* (1974) 731.
3 Sandberg, G., Söder, O., and Ernström, U., Int. Archs Allergy appl. Immun. *50* (1976) 374.
4 Pabst, R., and Pötschick, K., Immunology *50* (1983) 281.
5 Ernström, U., and Sandberg, G., Scand J. Haemat. *11* (1973) 275.
6 Ernström, U., and Sandberg, G., Acta physiol. Scand. *90* (1974) 202.
7 Davies, B. N., and Withrington, P. G., Pharmac. Rev. *25* (1973) 373.
8 Kudoh, G., Hoshi, K., and Murakami, T., Archvm histol. jap. *42* (1979) 169.
9 Schumacher, U., and Welsch, U., Am. J. Anat. *179* (1987) 356.
10 Reilly, F. D., Scanning Microscopy *5* (1991) 183.
11 Holmgren, S., and Nilsson, S., Eur. J. Pharmac. *32* (1975) 163.
12 Morse, M. A., Bell, L., and Rutlen, D. L., Acta physiol. scand. *138* (1990) 331.
13 Brunner, F., and Kukovetz, W. R., J. cardiovasc. Pharmac. *8* (1986) 712.
14 Yamada, S., Yamamura, H. I., and Roeske, W. R., Life Sci. *31* (1982) 1161.
15 Kirpekar, S. M., Prat, J. C., and Wakade, A. R., Naunyn-Schmiedeberg's Archs Pharmac. *287* (1975) 205.
16 Laduron, P., Nature *17* (1980) 287.
17 Strom, T. B., Lane, M. A., and George, K. J., Immunology *127* (1981) 705.
18 Flory, C. M., Dev. comp. Immun. *14* (1990) 283.
19 Takasaki, K., Tang, L. C., and Urabe, M., Jap. J. Pharmac. *29* (1979) 1.
20 Digges, K. G., McPherson, G. A., and Summers, R. J., J. Journal of Autonomic Pharmacology *1* (1981) 313.
21 Eriksson, B., and Hedfors, E., Scand. J. Haemat. *18* (1977) 121.
22 Steel, C. M., French, E. B., and Aitchison, W. R. C., Br. J. Haemat. *21* (1971) 413.
23 Van Tits, L. J. H., Michel, M. C., Grosse-Wilde, H., Happel, M., Eigler, F. W., Soliman, A., and Brode, O. E., Am. J. Physiol. *258* (1990) e191.
24 Chelazzi, G., Senaldi, G., Pinotti, G., Nicora, C., Rossi, D., and Mondelli, M., Boll. Ist. sieroter. milan. *66* (1987) 120.
25 Dürig, M., Landmann, R. M., and Harder, F., J. Lab. clin. Med. *104* (1984) 110.
26 Westermann, J., Schwinzer, R., Jecker, P., and Pabst, R., Scand. J. Haemat. *31* (1990) 327.
27 Alexeeva, T. A., and Ado, A. D., Allergol. Immunopath. (Madr) *14* (1968) 325.
28 Wazer, D. E., and Rotrosen, J., J. Pharm. Pharmac. *36* (1984) 853.
29 Costa, L. G., Kaylor, G., and Murphy, S. D., Immunopharmacology *16* (1988) 139.
30 Ado, A. D., and Dontsov, V. I., Bull. exp. Biol. Med. USSR *97* (1984) 202.
31 Chambers, D. A., Cohen, R. L., and Perlmamn, R. L., Neurochem. Int. *22* (1993) 95.
32 Saito, H., Am. J. Anat. *189* (1990) 213.
33 Zetterström, B. E. M., Hökfelt, T., Norberg, K. A., Olsson, P., Acta chir. scand. *139* (1973) 117.
34 Felten, D. L., Ackerman, K. D., Wiegand, S. J., and Felten, S. Y., J. Neurosci. Res. *18* (1987) 28.
35 Reilly, F. D., McCuskey, P. A., Miller, M. L., McCuskey, R. S., and Meineke, H. A., Tissue & Cell *11* (1979) 121.
36 Reilly, F. D., Experientia *41* (1985) 187.
37 Ulich, T. R., Dakay, E. B., Williams, J. H., Ni, R. X., Am. J. Path. *124* (1986) 53.
38 Asako, H., Kubes, P., Wallace, J., Gaginella, T., Wolf, R. E., and Granger, D. N., Am. J. Physiol. *262* (1992) g903.
39 Miller, S. C., Experientia *48* (1992) 674.